APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1989, p. 2287–2292
0099-2240/89/092287-06$02.00/0

Vol. 55, No. 9

Antibiotic Resistance of Gram-Negative Enteric Bacteria from Pigs in Three Herds with Different Histories of Antibiotic Exposure†

GLORIA GELLIN, BRUCE E. LANGLOIS,* KARL A. DAWSON, AND DEBRA K. AARON

Department of Animal Sciences, University of Kentucky, Lexington, Kentucky 40546-0215

Received 13 March 1989/Accepted 20 June 1989

The antibiotic resistance patterns of gram-negative fecal bacteria from pigs in three herds with different histories of antibiotic exposure were examined. In general, smaller proportions of antibiotic-resistant or multiply resistant fecal isolates ($P < 0.05$) were obtained from pigs in a herd not exposed to antimicrobial agents for 154 months than from pigs in a herd continuously exposed to antimicrobial agents at subtherapeutic doses or from pigs in a herd exposed only to therapeutic doses of antimicrobial agents. The proportions of antibiotic-resistant and multiply resistant strains were greater among isolates from pigs in the therapeutic herd than in the non-antibiotic-exposed herd ($P < 0.05$). The proportion of antibiotic-resistant isolates in the non-lactose-fermenting population was greater than that in the lactose-fermenting population, regardless of herd. The results suggest that any form of antimicrobial exposure will increase the prevalence of antimicrobial resistance and multiple resistance of fecal bacteria.

Concern has been expressed over the years that the continued use of subtherapeutic levels of antibiotics in feed will increase the risk of transfer of drug-resistant pathogenic bacteria from animals to humans (5, 9, 10, 22, 29, 30). Bacterial resistance to antibiotics has been shown to increase not only to the antibiotic being fed to the animals but also to other related antibiotics (2, 4, 10, 12, 13). Because of this increased resistance, it is thought that the use of subtherapeutic levels in animal feeds should be discontinued and only therapeutic treatment of animals should be allowed (10, 29, 30). There is little disagreement in the scientific community that usage of antibiotics will promote an increase in the proportion of resistant bacteria in the gastrointestinal tract (2, 4, 6–8, 11, 12, 19, 21, 22, 24, 25, 27). However, little information is available comparing the differential effects of therapeutic, subtherapeutic, or prophylactic uses of antimicrobial agents in selecting antibiotic resistance in animals (2, 3, 10, 13, 17).

This study was conducted to compare the antibiotic resistance of gram-negative fecal bacteria from pigs in three herds with different histories of antibiotic exposure. One herd had not been exposed to antimicrobial agents for 154 months (NAB herd), a second herd had routinely received subtherapeutic doses of antimicrobial agents and therapeutic doses when required to treat diseases (AB herd), and the third herd was given therapeutic antimicrobial agents when needed to treat diseases (TH herd).

MATERIALS AND METHODS

Source of isolates. Isolates were obtained from pigs in three herds with different histories of antibiotic exposure. The NAB and the AB herds have been described previously (11, 12, 14). The TH herd, in existence for 9 years at the time of sampling, was established by the Iowa Veterinary Medicine Research Institute. Iowa State University, Ames, as a completely closed and confined herd of specific-pathogen-free Yorkshire-Landrace and Hampshire crossbred pigs. Animals in the herd were delivered by cesarean section when the herd was initially established. Sows are bred by artificial insemination. The antibiotic-free diet was purchased from a commercial feed mill. The commercial feed was then supplemented with calcium and phosphorus at the facility before being fed to the pigs. The pigs were housed in four separate environmentally controlled buildings. The design of each building and the age distribution of pigs within each were similar. Caretakers and research scientists showered with a disinfectant and changed clothes before they entered the confinement area. In addition, they changed and disinfected their boots before going from one building to another. When therapeutic treatment was needed for control of infections, pigs were treated with 0.25 to 25 ml of 200,000 U of penicillin G procaine per ml and 0.25 g of dihydrostreptomycin base per ml for a maximum of 4 days. The amount of antibiotic and duration of antibiotic treatment depended on the weight of the pigs and the extent of the infection. Pigs in one building were being treated for *Pasturella* infections at the time of sampling. Approximately 18% of the 91 pigs had never been treated with antibiotics; 64.8% had been treated once, 12.1% had been treated twice, and 5.5% had been treated three times. All except two treated pigs were treated with antibiotics during the 11 months preceding sampling, and 52.7% were treated within 4 months of sampling.

Collection and storage of samples. Unless otherwise stated, all media used were obtained from Difco Laboratories (Detroit, Mich.) or BBL Microbiology Systems (Cockeysville, Md.) and incubation was at 35°C.

Rectal swab samples were obtained from 45 pigs in the NAB herd, from 51 pigs in the AB herd, and from 91 pigs in the TH herd.

Rectal swabs were streaked on MacConkey agar plates within 1 h of being collected. Five lactose-fermenting and up to five non-lactose-fermenting colonies were randomly picked from each plate after incubation, stabbed three times into Trypticase soy agar (BBL) deeps, incubated overnight, and then stored at 2°C until activated for antibiotic susceptibility testing. Isolates from the TH herd were picked to Trypticase soy agar deeps, held on ice during the 2-day trip to Lexington, Ky., and then incubated overnight.

Determination of lactose utilization. Each culture was activated by subculturing three times into brain heart infu-

* Corresponding author.

† Published with the approval of the Director of the Kentucky Agricultural Experiment Station as journal paper 89-5-45.

TABLE 1. Microbial groups based on lactose utilization obtained from pigs in three herds with different histories of antimicrobial exposure

Microbial group	No. (%) of isolates from following herd:		
	NAB	AB	TH
Lactose fermenter[a]	195 (79.9)	280 (71.4)	525 (76.3)
Slow lactose fermenter[b]	7 (2.9)		45 (6.5)
Non-lactose fermenter[c]	42 (17.2)	94 (2.4)	86 (12.5)
Mutant[d]		18 (4.6)	32 (4.7)

[a] Lactose utilized within 24 h.
[b] Lactose utilized between 24 and 72 h.
[c] Lactose not utilized within 72 h.
[d] Yellow papilla (lactose utilization) developed on top of non-lactose-fermenting colony between 24 and 72 h.

sion broth before streaking on Trypticase soy agar plates to check for purity. The ability of isolates to utilize lactose was determined on purple agar base containing 2% lactose after incubating plates for up to 72 h. Isolates were grouped according to their utilization of lactose as follows: (i) lactose fermenters if yellow zones surrounded growth on purple agar base containing 2% lactose within 24 h; (ii) slow lactose fermenters if lactose utilization occurred between 24 and 72 h; (iii) non-lactose fermenters if no color change occurred in the medium after 72 h; and (iv) mutants if a yellow papilla developed on top of a non-lactose-fermenting colony.

Antibiotic resistance. Custom MIC breakpoint plates (APBEL; Radiometer America, Westlake, Ohio) were used to determine the antibiotic susceptibilities of the isolates from the three herds to amikacin, ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, nalidixic acid, streptomycin, sulfamethoxazole-trimethoprim (SXT), sulfisoxazole, and tetracycline. Procedures, techniques, and interpretation of results were as recommended by the manufacturer for determining susceptibilities with a Sensititre MIC/ID automated system (Radiometer America, Westlake, Ohio) with an automatic inoculator. Isolates which showed intermediate resistance were considered to be resistant.

Statistical analysis. All isolates obtained from pigs in the same herd were combined for analysis. The percentages of isolates resistant to each antimicrobial agent and multiple antibiotic resistance were determined for isolates from each herd. Chi-square analysis (20) was used to determine the differences between proportions of all isolates, lactose fermenters and slow lactose fermenters combined, and non-lactose fermenters from the three herds which were resistant to ampicillin (16 μg/ml), carbenicillin (32 μg/ml), cephalothin (16 μg/ml), chloramphenicol (16 μg/ml), kanamycin (16 μg/ml), neomycin (8 μg/ml), nalidixic acid (16 μg/ml), streptomycin (16 μg/ml), SXT (8/152 μg/ml), sulfisoxazole (256 g/ml), and tetracycline (8 and 64 μg/ml) and the differences in the proportion of multiply resistant isolates.

RESULTS

A total of 1,324 gram-negative fecal isolates were examined in this study. The distribution of isolates from each herd according to lactose utilization is shown in Table 1. More than 71% of the isolates picked from each herd utilized lactose within 24 h. None of the isolates from the AB and NAB herds were classified as slow lactose fermenters and mutants, respectively.

Only one isolate from the TH herd was resistant to amikacin (32 μg/ml) or gentamicin (8 μg/ml). The proportions of all isolates, lactose fermenters, and non-lactose fermenters resistant to the other 11 antimicrobial agents are shown in Tables 2, 3, and 4.

Except for cephalothin and sulfisoxazole, a lower proportion of isolates from the NAB herd were resistant to the agents tested compared with isolates from the AB and TH herds (Table 2). A greater proportion of TH herd isolates were resistant to chloramphenicol, nalidixic acid, streptomycin, and SXT compared with AB herd ($P < 0.1$) and NAB herd ($P < 0.05$) isolates. The mean number of agents in the resistance patterns of isolates from the NAB herd was less than that for isolates from the AB and TH herds (Table 5). Less than 24% of NAB isolates were resistant to more than two agents, compared with 70.9 and 75.3% of AB and TH herd isolates, respectively. Isolates from the NAB herd showed resistance to a maximum of 6 agents, compared with a maximum of 9 and 11 agents for AB and TH herd isolates, respectively.

Antibiotic resistance of all isolates which utilized lactose within 72 h is shown in Table 3. The slow lactose fermenters were combined with isolates which utilized lactose within 24 h, since none of the isolates from the AB herd were considered to be slow lactose fermenters and the number of slow lactose fermenters from the other two herds was small.

TABLE 2. Antibiotic resistance of gram-negative fecal bacteria from pigs in three swine herds with different histories of antimicrobial exposure

Antimicrobial agent	Concn (μg/ml)	Resistance (%) in following herd:			Significant difference by[a]:
		NAB (n = 244)	AB (n = 392)	TH (n = 688)	
Ampicillin	16	10.7	50.5	25.7	AB > TH**, NAB**; TH > NAB**
Carbenicillin	32	10.2	50.5	22.8	AB > TH**, NAB**; TH > NAB**
Cephalothin	16	15.6	9.4	8.3	NAB > AB*, TH**
Chloramphenicol	16	0.4	1.0	2.8	TH > AB+, NAB*
Kanamycin	16	1.2	43.1	15.0	AB > TH**, NAB**; TH > NAB**
Nalidixic acid	16	3.7	5.9	8.7	TH > AB+, NAB**
Neomycin	8	3.3	38.8	14.0	AB > TH**, NAB**; TH > NAB**
Streptomycin	16	10.7	74.0	74.4	AB, TH > NAB**
SXT	8/152	0.4	5.1	10.9	TH > AB**, NAB**; AB > NAB**
Sulfisoxazole	256	98.8	98.5	95.8	AB, NAB > TH*
Tetracycline	8	23.8	99.0	80.4	AB > TH**, NAB**; TH > NAB**
Tetracycline	64	17.6	89.3	66.7	AB > TH**, NAB**; TH > NAB**

[a] Statistical significance symbols: +, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$.

TABLE 3. Antibiotic resistance of gram-negative lactose-fermenting fecal bacteria from pigs in three swine herds with different histories of antimicrobial exposure

Antimicrobial agent	Concn (μg/ml)	Resistance (%) in following herd:			Significant difference by[a]:
		NAB (n = 202)	AB (n = 280)	TH (n = 570)	
Ampicillin	16	0.5	36.4	25.1	AB > TH**, NAB**; TH > NAB**
Carbenicillin	32	0.5	36.8	23.0	AB > TH**, NAB**; TH > NAB**
Cephalothin	16	5.9	7.5	5.6	
Chloramphenicol	16	0	0.7	0.7	
Kanamycin	16	1.5	45.4	14.0	AB > TH**, NAB**; TH > NAB**
Nalidixic acid	16	3.5	4.3	2.8	
Neomycin	8	3.5	42.1	12.5	AB > TH**, NAB**; TH > NAB**
Streptomycin	16	12.4	74.3	74.0	AB, TH > NAB**
SXT	8/152	0	3.9	3.2	AB > NAB**; TH > NAB*
Sulfisoxazole	256	99.5	98.6	95.1	NAB > TH**; AB > TH*
Tetracycline	8	26.7	99.6	78.6	AB > TH**, NAB**; TH > NAB**
Tetracycline	64	20.3	86.1	67.0	AB > TH**, NAB**; TH > NAB**

[a] See Table 2, footnote *a*.

Except for sulfisoxazole, the proportion of resistant isolates tended to be greater in lactose fermenters obtained from the AB herd ($P < 0.01$). Proportions of resistant lactose fermenters from the TH herd generally were higher ($P < 0.05$) than those observed for lactose fermenters from the NAB herd. The mean number of agents in the resistance patterns of lactose fermenters from the NAB herd was less than that for lactose fermenters from the AB and TH herds (Table 5). Less than 16% of NAB lactose fermenters were resistant to more than two agents, compared with 64.7 and 73.3% of AB and TH herd lactose fermenters, respectively. Lactose fermenters from the NAB herd showed resistance to a maximum of five agents, compared with a maximum of seven and nine agents for AB and TH herd lactose fermenters, respectively.

The proportion of non-lactose fermenters resistant to cephalothin from the NAB herd ($P < 0.01$) was greater than that from the other two herds (Table 4). The proportion of non-lactose fermenters resistant to chloramphemicol, nalidixic acid, and SXT was higher ($P < 0.01$) for the TH herd than for the other two herds. Resistance to the other agents listed in Table 4 was greater ($P < 0.01$) for AB herd non-lactose fermenters. The proportion of resistant non-lactose fermenters from the TH herd generally was higher ($P < 0.05$) than observed for non-lactose fermenters from the NAB herd. While a greater proportion of non-lactose fermenters from the AB herd showed resistance to more than one agent (multiple resistance), only the difference in multiple resistance between the isolates from the AB and NAB herd non-lactose fermenters was significant (Table 5). A greater proportion of non-lactose fermenters than lactose fermenters were resistant to more than two agents (Table 5). Approximately 62% of NAB non-lactose fermenters were resistant to more than two agents, compared with 83 and 82.5% of AB and TH herd non-lactose fermenters, respectively. NAB herd non-lactose fermenters showed resistance to a maximum of 6 agents, compared with a maximum of 8 and 11 agents for AB and TH herd non-lactose fermenters, respectively.

All slow lactose fermenters showed multiple resistance. The 7 slow lactose fermenters from the NAB herd were resistant to a maximum of four agents, compared with a maximum of nine agents for the 45 slow lactose fermenters from the TH herd. However, the small number of slow lactose fermenters observed may account for the nonsignificant difference in multiple resistance obtained between the NAB and TH herds. The slow lactose fermenters from the NAB herd showed resistance to ampicillin (14.3%), cephalothin (100%), nalidixic acid (42.9%), sulfisoxazole (100%), and tetracycline, 8 μg/ml (42.9%). The slow lactose ferment-

TABLE 4. Antibiotic resistance of gram-negative non-lactose-fermenting fecal bacteria from pigs in three swine herds with different histories of antimicrobial exposure

Antimicrobial agent	Concn (μg/ml)	Resistance (%) in following herd:			Significant difference by[a]:
		NAB (n = 42)	AB (n = 94)	TH (n = 86)	
Ampicillin	16	59.5	83.0	30.2	AB > TH**, NAB**; NAB > TH**
Carbenicillin	32	57.1	81.9	20.9	AB > TH**, NAB**; NAB > TH**
Cephalothin	16	61.9	14.9	22.1	NAB > AB**, TH**
Chloramphenicol	16	2.4	2.1	16.3	TH > AB**, NAB*
Kanamycin	16	0	30.9	24.4	AB, TH > NAB**
Nalidixic acid	16	4.8	7.4	36.0	TH > AB** NAB**
Neomycin	8	2.4	26.6	25.6	AB, TH > NAB**
Streptomycin	16	2.4	72.3	70.9	AB, TH > NAB**
SXT	8/152	2.4	3.2	48.8	TH > AB**, NAB**
Sulfisoxazole	256	95.2	97.9	98.8	
Tetracycline	8	9.5	96.8	84.9	AB > TH**, NAB**; TH > NAB**
Tetracycline	64	4.8	96.8	53.5	AB > TH**, NAB**; TH > NAB**

[a] See Table 2, footnote *a*.

TABLE 5. Resistance to multiple antimicrobial agents of enteric bacteria from feces of pigs in three herds with different histories of antimicrobial exposure

Herd	Microbial group[a]	No. of isolates	% of isolates resistant to the following number of agents:											MMR[b]
			0	1	2	3	4	5	6	7	8	9	10	11	
NAB	All	244	0.4	61.1	14.8	10.2	10.7	2.5	0.4						1.79
	LF	202	0.5	66.9	16.8	11.4	3.0	1.5							1.54
	NLF	42		33.3	4.8	4.8	47.6	7.1	2.4						2.98
AB	All	382	0.8	13.3	15.3	7.7	30.4	14.8	12.8	4.1	0.8	0.3			4.76
	LF	280	1.1	15.0	19.3	8.6	27.9	13.2	12.1	2.9					4.50
	NLF	94		10.6	6.4	3.2	42.6	17.0	13.8	5.3	1.1				5.17
	Mutant	18				16.2	5.6	27.8	16.7	16.7	11.1	5.6			6.67
TH	All	688	0.9	8.3	15.6	33.3	12.1	20.9	2.9	3.1	0.7	1.3	0.7	0.3	3.59
	LF	570	1.1	9.6	16.1	36.0	10.0	22.1	2.5	1.9	0.4	0.4			3.35
	NLF	86		2.3	15.1	20.9	19.8	11.6	4.7	8.1	3.5	5.8	5.8	2.3	4.81
	Mutant	32			6.3	18.8	8.1	25.0	6.3	9.4					4.61

[a] All, All isolates tested; LF, lactose fermenter; NLF, non-lactose fermenter. See Table 1, footnotes *a*, *c*, and *d*.
[b] MMR, Mean number of isolates resistant to the following (in micrograms per milliliter): amikacin, 32; ampicillin, 16; carbenicillin, 32; cephalothin, 16; chloramphenicol, 16; gentamicin, 8; kanamycin, 16; nalidixic acid, 16; neomycin, 8; streptomycin, 16; SXT, 8/152; sulfisoxazole, 256; and tetracycline, 8.

ers from the TH herd showed resistance to 11 of the 13 antimicrobial agents tested. The TH herd slow lactose fermenters were resistant to ampicillin (8.9%), carbenicillin (8.9%), cephalothin (13.3%), chloramphenicol (2.2%), kanamycin (13.3%), nalidixic acid (28.9%), neomycin (13.3%), streptomycin (68.9%), SXT (24.4%), sulfisoxazole (100%), and both concentrations of tetracycline (97.8%).

The mean number of antimicrobial agents in the resistance pattern was greater ($P < 0.01$) in mutant isolates from the AB herd than in those from the TH herd. All AB herd mutants were resistant to three or more agents and some were resistant to as many as nine agents. The TH herd mutants were resistant to two or more agents and up to a maximum of seven agents. The 18 AB herd mutants were resistant to ampicillin (100%), carbenicillin (100%), cephalothin (11.1%), kanamycin (72.2%), nalidixic acid (22.2%), neomycin (50%), streptomycin (77.7%), SXT (33.3%), sulfisoxazole (100%), and both concentrations of tetracycline (100%). The 32 TH herd mutants were resistant to ampicillin (25%), carbenicillin (25%), cephalothin (18.8%), chloramphenicol (3.1%), kanamycin (6.3%), nalidixic acid (40.6%), neomycin (9.4%), streptomycin (90.6%), SXT (46%), sulfisoxazole (100%), and both concentrations of tetracycline (100 and 96.9%).

The greatest number of antimicrobial resistance patterns was obtained for the TH herd isolates (68), which was 20 and 47 more than obtained for the AB and NAB herd isolates, respectively.

DISCUSSION

In general, antibiotic resistance and multiple antibiotic resistance were greater ($P < 0.05$) for isolates from pigs in the AB herd than for isolates from pigs in the NAB or TH herd. Antimicrobial resistance and multiple resistance were greater ($P < 0.05$) among isolates from pigs in the TH herd than in the NAB herd.

The results of this study support those obtained in earlier studies which suggest that any form of antimicrobial exposure increases the prevalence of antibiotic resistance (2, 3, 17). Langlois et al. (17) found that the proportion of antibiotic resistance fecal bacteria was greater in pigs given therapeutic dosages of chlortetracycline (CTC) for 14 days than in pigs given subtherapeutic dosages for 85 days. These authors found less of a decrease in antibiotic-resistant bacteria in pigs given therapeutic dosages of CTC compared with pigs fed continuous subtherapeutic dosages of CTC once the CTC was withdrawn. Sogaard (28) reported that 74% of *Escherichia coli* isolates from pigs given therapeutic dosages of antibiotics showed resistance to one or more antibiotics, compared with 53% of *E. coli* isolates from pigs given no antibiotics. Thus, the therapeutic use of antibiotics resulted in a 21% increase in the proportion of *E. coli* isolates resistant to various antibiotics.

Resistance to kanamycin and neomycin in the AB herd isolates may be related to the feeding of neoterramycin (neomycin and oxytetracycline) to lactating sows and the routine feeding of CTC to all pigs in the AB herd. Neomycin, kanamycin, oxytetracycline, and CTC are all aminoglycoside antibiotics and cross resistance may occur (1). Many studies have shown that therapeutic or subtherapeutic use of antibiotics will increase the frequency of resistant bacteria in the intestinal tract (2, 3, 6, 7, 11, 12, 17, 18, 24, 25, 28).

The high resistance to ampicillin and carbenicillin in the AB herd isolates is possibly a result of feeding low levels of penicillin to piglets up to 15 kg of body weight. Since ampicillin and carbenicillin are semisynthetic derivatives of penicillin, cross resistance could have occurred, causing a carry-over of resistance from piglets to pigs in the AB herd (1, 23).

The therapeutic use of dihydrostreptomycin in the AB and TH herds may be the reason that more than 70% of the isolates were resistant to streptomycin. Mercer et al. (21) found that the percentage of isolates resistant to a specific antibiotic increased when the antibiotic was fed. Other researchers have observed that resistance may develop to antimicrobial agents in addition to the one being used. Thus, the increase in multiple resistance observed in isolates from the AB and TH herds may be due in part to the transfer of R plasmid originating from the use of subtherapeutic or therapeutic antimicrobial agents.

The high proportion of tetracycline-resistant isolates from the AB herd was expected, since continuous subtherapeutic levels of CTC have been fed to pigs in this herd for more than 13 years. The high proportion of tetracycline-resistant isolates from the TH herd was not expected, since pigs in this herd have not been "knowingly" exposed to tetracyclines.

Apparently the therapeutic use of penicillin G procaine and dihydrostreptomycin also resulted in the development of resistance to tetracycline. Since pigs in both the AB and TH herds were housed in an enclosed environment, antibiotic-resistant bacteria could easily be passed between the bacterial flora of the pigs. Langlois et al. (16) reported a higher percentage of bacteria resistant to tetracycline in an enclosed housing unit (63.5%) when compared with those on pasture (24.2%). Linton et al. (19) found similar results when calves fed an antibiotic-free diet were placed in the same housing unit as calves fed an antibiotic-supplemented diet. The calves fed the antibiotic-free diet showed the same resistance pattern and serotype as the calves fed the antibiotic-supplemented diet.

The lower percentage of tetracycline-resistant isolates in the NAB herd may be attributed to the withdrawal of all antibiotics in this herd for 154 months. However, the decrease to lower levels of antibiotic resistance in fecal coliforms has taken a long time in the NAB herd. Residual effects of antibiotics used before withdrawal appear to still be occurring even after withdrawal for 154 months. The short- or long-term use of antibiotics is thought to increase the number of resistant bacteria in the gastrointestinal tract and they can be passed on from one generation to the next. Linton (18) theorized that once a resistant organism colonizes the intestinal tract, it may eventually dominate the microflora. Smith (26) also believes that once a resistant strain occurs, it can compete with susceptible organisms. Thus, it appears that once R plasmids and transposons are established in the fecal flora, they may be retained by selective advantages other than those provided by antimicrobial usage. Persistence of R-plasmid-containing strains also may be facilitated by environmental factors. Resistant strains which initially contaminated the living quarters of the animals may be subsequently reintroduced to a new generation of animals. However, this does not explain why resistance is less in pigs over 6 months of age or in pigs, regardless of age, housed on pasture (16).

In general, antimicrobial resistance and multiple antimicrobial resistance were greater in non-lactose fermenters than in lactose fermenters, regardless of herd. Little information is available concerning the role that non-lactose fermenters play in the transfer of antimicrobial resistance or the prevalence of transfer between lactose fermenters and non-lactose fermenters. For years, antibiotic resistance of fecal coliforms has been used to indicate the development of antimicrobial resistance in the intestinal microflora due to the use of antimicrobial agents in animals. However, the results obtained in this study raise the question whether coliforms are the best microbial group to use to evaluate antimicrobial resistance in animals and to determine potential human health hazards.

While other studies (13–16) suggest that a number of factors can influence the prevalence of antibiotic-resistant strains in the fecal material of swine, this study suggests that both subtherapeutic and therapeutic usage of antimicrobial agents will result in increased proportions of drug-resistant and multiply resistant fecal bacteria. However, reversion to lower levels of antibiotic resistance and multiple resistance will take a long time once all uses of antimicrobial agents have been withdrawn.

ACKNOWLEDGMENTS

We express our appreciation to R. F. Ross, M. Wannemuehler, and Tom Carlson for their assistance in obtaining fecal isolates from the swine maintained at the Veterinary Medicine Research Institute at Iowa State University, to LeeAnne Jackson and Katherine Akers for their assistance in analyzing the isolates, and to Sara Jenkins for the statistical analysis.

LITERATURE CITED

1. **Brander, G. C., D. M. Pugh, and R. J. Bywater (ed.).** 1982. Veterinary applied pharmacology and therapeutics, 4th ed. Cassell Ltd., London.
2. **Dawson, K. A., B. E. Langlois, T. S. Stahly, and G. L. Cromwell.** 1983. Multiple antibiotic resistance in fecal, cecal and colonic coliforms from pigs fed therapeutic and subtherapeutic concentrations of chlortetracycline. J. Anim. Sci. **57**:1225–1234.
3. **Dawson, K. A., B. E. Langlois, T. S. Stahly, and G. L. Cromwell.** 1984. Antibiotic resistance in anaerobic and coliform bacteria from the intestinal tract of swine fed therapeutic and subtherapeutic concentrations of chlortetracycline. J. Anim. Sci. **58**:123–131.
4. **Dawson, K. A., B. E. Langlois, T. S. Stahly, and G. L. Cromwell.** 1984. Some characteristics and antibiotic resistance of anaerobic bacteria from the ceca and colons of pigs fed chlortetracycline-containing and unmedicated diets. Appl. Environ. Microbiol. **47**:210–212.
5. **Falkow, S.** 1975. Infectious multiple drug resistance. Pion Limited, London.
6. **Frolich, A., E. Kvarnfors, I. Manson, and A. Simonsson.** 1974. Antibiotic additives in sow's diet—effects on production and intestinal flora. Acta Agric. Scand. **24**:273–285.
7. **Fuller, R., L. G. M. Newland, C. A. E. Briggs, R. Braude, and K. G. Mitchell.** 1960. The normal intestinal flora of the pig. IV. The effect of dietary supplements of penicillin, chlortetracycline or copper sulphate on the fecal flora. J. Appl. Bacteriol. **23**:195–205.
8. **Gaines, S. A., L. D. Rollins, R. D. Williams, and M. Selwyn.** 1980. Effect of penicillin and virginiamycin on drug resistance in lactose-fermenting enteric flora. Antimicrob. Agents Chemother. **17**:428–433.
9. **Guest, G. B.** 1976. Status of FDA's program on the use of antibiotics in animal feeds. J. Anim. Sci. **42**:1052–1057.
10. **Institute of Medicine.** 1988. Human health risks with the subtherapeutic use of penicillin and tetracyclines in animal feed. National Academy Press, Washington, D.C.
11. **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**:1369–1382.
12. **Langlois, B. E., G. L. Cromwell, and V. W. Hays.** 1978. Influence of type of antibiotic and length of antibiotic feeding period on performance and persistence of antibiotic resistant enteric bacteria in growing-finishing swine. J. Anim. Sci. **46**: 1383–1396.
13. **Langlois, B. E., G. L. Cromwell, T. S. Stahly, K. A. Dawson, and V. W. Hays.** 1983. Antibiotic resistance of fecal coliforms after long-term withdrawal of therapeutic and subtherapeutic antibiotic use in a swine herd. Appl. Environ. Microbiol. **46**:1433–1434.
14. **Langlois, B. E., K. A. Dawson, G. L. Cromwell, and T. S. Stahly.** 1986. Antibiotic resistance in pigs following a 13 year ban. J. Anim. Sci. **62**(Suppl. 3):18–32.
15. **Langlois, B. E., K. A. Dawson, I. Leak, and D. K. Aaron.** 1988. Antimicrobial resistance of fecal coliforms from pigs in a herd not exposed to antimicrobial agents for 126 months. Vet. Microbiol. **18**:147–153.
16. **Langlois, B. E., K. A. Dawson, I. Leak, and D. K. Aaron.** 1988. Effect of age and housing location on antibiotic resistance of fecal coliforms from pigs in a non-antibiotic-exposed herd. Appl. Environ. Microbiol. **54**:1341–1344.
17. **Langlois, B. E., K. A. Dawson, T. S. Stahly, and G. L. Cromwell.** 1984. Antibiotic resistance of fecal coliforms from swine fed subtherapeutic and therapeutic levels of chlortetracycline. J. Anim. Sci. **58**:666–674.
18. **Linton, A. H.** 1977. Antibiotics, animal and man—an appraisal

of a contentious subject, p. 315–343. *In* M. Woodbine (ed.), Antibiotics and antibiosis in agriculture. Butterworths, Inc., Woburn, Mass.
19. **Linton, A. H., K. Howe, and A. D. Osborne.** 1975. The effects of feeding tetracycline, nitrovin and quindoxin on the drug resistance of coli-aerogenes bacteria from calves and pigs. J. Appl. Bacteriol. **38**:255–275.
20. **Matthews, D. E., and V. T. Farewell.** 1985. Using and understanding medical statistics. Karge, New York.
21. **Mercer, H. D., D. Pocurull, S. Gaines, S. Wilson, and J. V. Bennett.** 1971. Characteristics of antimicrobial resistance of *Escherichia coli* from animals: relationship to veterinary and management use of antimicrobial agents. Appl. Microbiol. **22:** 700–705.
22. **Novick, R. P.** 1981. The development and spread of antibiotic-resistant bacteria as a consequence of feeding antibiotics to livestock. Ann. N.Y. Acad. Sci. **368**:23–59.
23. **Russell, A. D., and L. B. Quesnel (ed.).** 1983. Antibiotics: assessment of antimicrobial activity and resistance, p. 1–17. Academic Press, Inc., New York.
24. **Siegel, D., W. G. Huber, and F. Enloe.** 1974. Continuous non-therapeutic use of antibacterial drugs in feed and drug resistance of the gram-negative enteric florae of food-producing animals. Antimicrob. Agents Chemother. **6**:697–701.
25. **Smith, H. W.** 1971. The effects of the use of antibiotics on the emergence of drug-resistant bacteria in animals. Adv. Vet. Sci. Comp. Med. **15**:67–100.
26. **Smith, H. W.** 1970. The transfer of antibiotic resistance between strains of enterobacteria in children, calves and pigs. J. Med. Microbiol. **3**:165–180.
27. **Smith, H. W., and W. E. Crabb.** 1957. The effect of the continuous administration of diets containing low levels of tetracycline 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.
28. **Sogaard, H.** 1973. Incidence of drug resistance and transmissible R factors in strains of *E. coli* from faeces of healthy pigs. Acta Vet. Scand. **14**:381–391.
29. **Swann, M. M.** 1969. Joint committee on the use of antibiotics in animal husbandry and veterinary medicine. Her Majesty's Stationery Office, London.
30. **Van Houweling, C. D.** 1972. Report of the FDA Task Force on the use of antibiotics in animal feeds. Food and Drug Administration, Rockville, Md.