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Multiple Antibiotic Resistance Patterns of *Escherichia coli* Isolates from Swine Farms

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Antibiotic resistance of *Escherichia coli* from sows and pigs was determined to compare patterns between pigs of various ages and degrees of antibiotic use. Resistance patterns differed between farm types and pigs of differing ages, indicating that pig age and degree of antibiotic use affect resistance of fecal *E. coli*.

Antibiotics are used in livestock systems to combat disease and improve animal productivity. Feed-based antibiotics consistently benefit productivity, increasing the ability of farms to maintain profitable margins. Benefits of antibiotic use include improved growth (7, 20), decreased nitrogen excretion and thus reduced environmental impact (17), and decreased pathogen loads (11).

Contrasting the above benefits are suggestions that agricultural use of antibiotics may be partly responsible for the emergence of drug-resistant organisms (4). While a number of studies have been conducted and conferences have been assembled to address this issue (3, 8), a lack of data continues to hamper efforts to devise solutions. Information on resistance prevalence and effect of farm management is especially lacking. Much of the current data are derived from clinical isolates, and as such, these data may be biased by age and condition of the animals and level of antibiotic use. So that more definitive recommendations can be devised, it is important that confounding factors be characterized. This study was designed to determine effects of pig age and level of antibiotic use on single and multiple antibiotic resistance patterns.

Ten swine farms in various regions of Tennessee were selected for this study. All farms were typical of U.S. production, with sizes ranging from approximately 1,500 to 10,000 pigs produced annually. Farms were classified as low antibiotic use (LU) ($n = 3$) if subtherapeutic feed-based antibiotics were not used or if only subtherapeutic concentrations of tetracyclines were used for brief periods. Farms classified as high antibiotic use (HU) ($n = 7$) routinely used subtherapeutic feed-based antibiotics and/or injectable antibiotics.

Five sows were randomly selected from each farm, and fecal material was collected via rectal swab at 7 days postpartum. Swabs were also obtained from five pigs from each test sow. Pigs were identified by ear notches so that additional samples could be collected at 35 and 63 days of age. *Escherichia coli* was isolated by culture on lactose MacConkey agar prior to confirmation by biochemical analysis (API20; Vitek bioMerieux, Syosset, N.Y.). Isolates were subjected to standardized disk diffusion tests (5, 16) against apramycin (15 μg), carbadox (20 μg), gentamicin (10 μg), neomycin (30 μg), and oxytetracycline (30 μg). Isolates were determined to be resistant if zone sizes were less than 14 mm for apramycin, 11 mm for carbadox, 14 mm for gentamicin, 16 mm for neomycin, and 18 mm for oxytetracycline.

Data on resistance to individual drugs were summarized by establishment of multiple-drug resistance groups and analyzed in two- and three-way contingency tables to test differences in frequencies of multiple-drug resistance groups across farms, pigs or sows, and time. Fisher's exact test and Cochran-Mantel-Haenszel tests (1) were used where appropriate, using SAS software (18).

Table 1 provides a summary of confirmed use of test antibiotics for each farm group. While farms were separated into LU and HU categories based on degree of antibiotic use, we are unable to provide a more detailed history of use for each antibiotic since some producers on HU farms could not reliably provide detailed drug use history beyond a few months prior to the study. We thus felt that specific indications on a farm-by-farm basis were less reliable and might result in erroneous conclusions.

On LU farms the incidence of multiple resistance generally remained constant throughout the various growth phases. On HU farms, incidence of multiple resistance was greatest ($P < 0.001$) in *E. coli* from pigs at 35 days of age (Table 2), except for patterns including neomycin and oxytetracycline, which increased at 63 days of age. In general, the incidence of resistance was lower at all pig ages on LU farms than on HU farms. No *E. coli* isolates from LU farms showed single resistance to apramycin (A), and only a few such isolates were noted from HU farms. Instead, the majority of apramycin resistance occurred as part of a multiple resistance pattern. Similarly, few isolates were found to be resistant only to neomycin (N); instead, neomycin resistance generally occurred as part of a

TABLE 1. Use of test antibiotics within 1 year previous to survey

Antibiotic	Method of presentation	Confirmed use[a] on: HU farms	LU farms
Apramycin	Feed (sulfate form, 165 mg/kg of feed)	×	
Carbadox	Feed (55 mg/kg of feed)	×	
Gentamicin	Water (sulfate form, 6.6 mg/liter of drinking water)	×	
Neomycin	Water (sulfate form, 22 mg/kg of body weight)	×	
Oxytetracycline	Feed (110 mg/kg of feed)	×	×
Oxytetracycline	Injected (HCl form, 10 mg/kg of body weight)	×	

[a] Confirmed use on one or more farms within the group.

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TABLE 2. Incidence of single and multiple resistance to tested antibiotics in fecal *E. coli* from sows and pigs of various ages exposed to high and low antibiotic usage

Drug and resistance pattern	% of isolates with indicated resistance pattern from animals and farm type (*n*)[a]							
	Sows		Pigs, 7 days		Pigs, 35 days		Pigs, 63 days	
	HU (322)	LU (140)	HU (1,731)	LU (747)	HU (1,683)	LU (729)	HU (999)	LU (407)
Apramycin								
Single	0	0	0	0	0	0	0	0
Multiple	0.3	0.7	8.2	9.2	60.9	7.1	29.5	5.8
No resistance	99.7	99.3	91.8	90.8	39.1	92.9	70.5	94.2
Carbadox								
Single	0	0.7	0.1	0.9	1.7	0	0	0.2
Multiple	17.7	37.8	35	17.8	55.3	17.1	47.3	20.6
No resistance	82.3	61.5	64.9	81.3	43.0	82.9	52.7	79.2
Gentamicin								
Single	0	3.6	0.5	5.9	0.1	6.4	0	0
Multiple	74.8	82.8	73.3	75.8	91.9	71.0	85.9	72.5
No resistance	25.2	13.6	26.2	18.3	8.0	22.6	14.1	27.5
Neomycin								
Single	0	0	0.2	0	0	0	0	0
Multiple	56.2	53.6	55.2	44.6	54.8	41.7	64.6	34.3
No resistance	43.8	46.4	44.6	55.4	45.2	58.3	35.4	65.7
Tetracycline								
Single	17.4	5.0	16.7	10.8	2.9	11.0	5.2	22.6
Multiple	81.7	79.3	79.5	71.8	91.4	78.9	93.6	76.9
No resistance	0.9	15.7	3.8	17.4	5.7	10.1	1.2	0.5

[a] The approximate least significant difference between percentages ranges from 3 to 8.

TABLE 3. Incidence of single and multiple resistance patterns in fecal *E. coli* from sows and pigs exposed to high and low antibiotic use

Resistance pattern	% of isolates with indicated resistance pattern from animals and farm type (*n*)[a]							
	Sows		Pigs, 7 days		Pigs, 35 days		Pigs, 63 days	
	HU (322)	LU (140)	HU (1,731)	LU (747)	HU (1,683)	LU (729)	HU (999)	LU (407)
A-C-G	0	0	0	0	1.5	0	0	0
A-C-G-N	0	0	0	0	1.2	0	0	0
A-C-G-N-O	0.3	0	1.3	0.1	19.7	0.1	14.8	2.0
A-C-G-O	0	0	0.6	0.1	19.2	0.3	3.2	2.7
A-C-N-O	0	0.7	0	0	0.1	0	0	0
A-C-O	0	0	0	0	0.1	0	0	0
A-G	0	0	0	0.1	0.1	0	0	0
A-G-N	0	0	0	1.5	0	0	0	0
A-G-N-O	0	0.7	5.4	6.6	13.0	0.7	7.6	0.3
A-G-O	0	0	0.9	0.8	6	5.9	3.7	1.0
A-N-O	0	0	0	0	0	0	0.1	0
A-O	0	0	0	0.9	0.1	0.1	0.1	0
C-G	0	0.7	0.1	0	0.4	0	0.3	0
C-G-N	0	0.7	0.1	2.1	0.3	0.1	0.3	0
C-G-N-O	10.6	20.7	0.1	7.4	6.1	5.4	13.8	5.9
C-G-O	3.8	14.3	7.9	6.3	4.3	4.0	10.5	7.4
C-N	0	0	3.4	0	0	0	0	0
C-N-O	0.9	0	0.1	0.13	0.6	0.4	1.9	0.3
C-O	2.2	1.4	0.4	1.6	1.8	6.9	2.5	2.5
G-N	0.3	5.0	1.0	3.5	0.2	2.2	0.1	0.3
G-N-O	40.1	25.0	33.1	21.8	13.0	30.0	22.1	23.8
G-O	19.9	15.7	19.5	25.4	6.8	22.4	9.4	29.2
N-O	4.0	1.4	5.8	1.1	0.7	2.7	3.8	2.0
A	0	0	0	0	0	0	0	0
C	0.1	0.7	0.1	0.9	1.7	0	0	0.3
G	0	3.6	0.6	5.9	0.1	6.5	0	0
N	0	0	0.2	0	0	0	0	0
O	17.4	5.0	16.7	10.8	2.9	11.0	5.2	22.6
Sensitive to all	0.6	5.0	1.6	3.35	0.1	1.4	0.5	0

[a] Standard errors were approximately 10% of the percentages, so least significant differences ranged from approximately 1 to 10, with higher values occurring with the larger percentages.

multiple resistance pattern. In contrast, oxytetracycline (O) resistance was often found as a single resistance. The greatest apramycin resistance was found in 35-day-old pigs on all seven HU farms, with a similar pattern observed for gentamicin on six of the seven HU farms (data not shown). In contrast, apramycin and gentamicin resistance did not increase at that pig age on any of the 3 LU farms. On LU farms, the most common combinations included C-G-N-O, C-G-O, and G-N-O (Table 3). These same patterns were found on HU farms, and in most cases the incidence was greater than that on LU farms. We observed a marked increase in the incidence of A-C-G-N-O, A-C-G-O, and A-G-N-O multiple resistance patterns in *E. coli* from HU farms in pigs at 35 days of age.

Whereas survey data on resistance are commonly reported, age of the animals is generally not taken into account. Our data indicate that age is an important factor affecting resistance in swine. The increased incidence of resistance noted in nursery pigs may be a reflection of increased antibiotic use at that time, and may also reflect the increased colonization by pathogens that occurs postweaning (14, 15), in which resistance may occur more commonly than in commensal organisms.

Cross-resistance within the aminoglycoside group was common. One of the most common combinations was N-G-O. The two aminoglycosides in this pattern, neomycin and gentamicin, share more commonalities in structure and function than with apramycin. *E. coli* from pigs had a greater incidence of resistance to A-G-N-O, while *E. coli* from sows had greater resistance to C-G-N-O. Apramycin was a common feed additive reportedly used on HU farms in nursery-age pigs; these data may indicate that transfer of organisms occurs between nurseries and farrowing (birthing) rooms. If that is the case, the contamination appears to have a greater effect in pigs than in sows. This may be due to resistant bacteria that specifically colonize the young pig or a greater resistance by the sow to invasion by bacteria from an outside source. This would support other reports suggesting that pigs, and not sows, are the primary sources of *E. coli* in farrowing barns (2, 6). Hinton and Linton (9) and Katouli et al. (10) determined that young pigs are able to maintain a unique microfloral population even while in close contact with the sow. Other investigators also observed higher percentages of resistant bacteria in young mammals than in adults (12, 13, 19, 22). Because younger swine are at a greater risk of contracting disease (21), they likely represent the most common source of clinical isolates, which in turn are more likely to be the source of resistance. It is evident from this study that resistance data from young pigs should not be extrapolated for estimates in market-age swine, which have the greatest relevance to pork products. Further studies to determine other factors affecting resistant bacteria in livestock systems appear warranted.

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