Changes in the Prevalence of Antimicrobial Resistance in a Vertically Integrated Veal Calf Production System

Honors Research Thesis

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ABSTRACT

Antimicrobial resistance is a public health concern for both human and veterinary medicine. In food animal production systems, medically important antimicrobials are used for both prophylactic and therapeutic purposes; therefore, food animals have the potential to serve as a reservoir for antimicrobial resistant bacteria. Previous research has shown an uneven distribution of resistance with a higher prevalence within young animals; however, limited research has addressed antimicrobial resistance within veal production systems. Vertically integrated veal production systems provide a unique opportunity to study the transmission of resistance through the food supply. The study's objective was to estimate the prevalence of antimicrobial resistant *Escherichia coli* within different stages of a vertically integrated veal production system. A total of 377 fecal samples were collected from nine different calf cohorts on six farms, where the average age was 69 days (range: 8-115). Four of these cohorts were followed to harvest for additional sample collection. At harvest, a total of 159 fecal samples, 161 pre-evisceration and 150 post-evisceration carcass swabs were collected. A single *E. coli* isolate from the samples was subjected to twelve antimicrobials using Kirby-Bauer disk diffusion assays. Zones of growth inhibition were measured for each antimicrobial and classified as resistant or susceptible based on CLSI interpretive criteria. *E. coli* Isolates were obtained from 100% of fecal samples, 52% (84/161) of pre-evisceration swabs and 16% (24/150) of post-evisceration swabs. Greater than 98% (372/377) of isolates obtained from farm fecal samples were resistant to two or more classes of antimicrobials. A decrease in resistance was seen at harvest where only 46.9% (73/159), 69.0% (58/84), and 29.2% (7/24) of isolates from fecal samples, pre-evisceration and post-evisceration carcass swabs, were resistant to two or more antimicrobials. These results

provide insight to the current prevalence of resistance among the production system and the opportunity for further research to determine factors affecting the prevalence of resistance.

INTRODUCTION

For more than 50 years, antimicrobial drugs have been used for health benefits in both human and veterinary medicine; however, development of bacterial resistance to antimicrobials is an increasing public health concern (1). According to the Centers for Disease Control and Prevention, over 2 million people in the United Stated become infected with bacteria that are resistant to antimicrobials, leading to over 23,000 deaths per year (2). Although the percentage of these resistant infections attributable to antimicrobial use in livestock production is unknown, it is estimated that 80% of antibiotics sold in the United States are used in animal production systems (7). These antibiotics are used for disease prevention and treatment as well as growth promotion. In addition to treating sick or injured animals, antimicrobials are often given at low levels as a preventative measure to minimize the spread of disease during vulnerable times in the production process. Furthermore, to increase feed efficiency, enhance growth rates and maximize production potential, antibiotics are often given at low doses to select for beneficial microorganisms within the gut.

Food producing animals are colonized with bacteria that are commensals or opportunistic pathogens. Antimicrobials exert a selective pressure for bacteria capable of withstanding their effects, and may inadvertently create a reservoir of resistant bacteria (6). As the prevalence of antimicrobial resistance increases in food animals, the concern of transmission to humans and the food supply increases. Transmission may occur through direct contact with animals or contamination of animal food products. Animal products can be contaminated on farm or during the harvest process through contact with intestinal contents, the animal hide or the environment.

Although transmission is often associated with known pathogenic bacteria, such as *Salmonella*, it may also occur through commensal organisms, such as *Escherichia coli*. Commensal organisms are more prevalent than pathogens, and often contain mobile resistance elements which can be transmitted to other bacteria and pathogens through horizontal gene transfer (5).

Since *E. coli* is a known commensal organism with the ability to harbor and transfer resistance genes, it is often used as an indicator organism to estimate the prevalence of antimicrobial resistance (AMR). Previous research had demonstrated that the prevalence of AMR bacteria is not equally distributed across all age groups in cattle (5). It is know that younger animals usually have higher levels of resistant bacteria, independent of exposure to antimicrobial drugs (5). Since veal calves enter the food system at a young age, vertically integrated veal production systems provide a unique opportunity to study the transmission of resistance through the food supply.

The objective of this study was to estimate the prevalence of antimicrobial resistant *E. coli* at different time points in a vertically integrated veal calf production system. In addition, the study aimed to provide preliminary data for future studies investigating factors that may influence resistance in the veal calf production system. The hypothesis for the study was that the prevalence of antimicrobial resistance *E. coli* would decrease as calves move through the production system.

MATERIALS AND METHODS

For this study, a longitudinal observational design was utilized. Nine calf cohorts from five different farms were sampled on farm, and four of the calf cohorts were followed to harvest where additional samples were obtained. On farm, fecal samples were collected from approximately 42 calves per farm using rectal palpation techniques. Where necessary, sampled

calves were given ear tags so they could later be identified at harvest. From the four cohorts followed to harvest, sterile cotton swabs were used to collect additional fecal samples from the rectum of the gastrointestinal tract following evisceration. In addition, 10 inch by 10 inch pre-evisceration and post-evisceration carcass swabs were collected from behind the shoulder using sterile sponges following USDA protocol (3). The sterile sponges were saturated with 25 mL of tryptic soy broth on-site prior to swabbing the carcass. The pre-evisceration carcass swab was obtained from the left side of the carcass immediately following the dehiding of the carcass. The post-evisceration carcass swabs were collected in the cold room from the right side of the carcass following the lactic acid wash.

Collected samples were brought to the Department of Veterinary Preventative Medicine, The Ohio State University for processing. A cotton swab from each fecal sample was plated onto MacConkey agar for isolation of gram-negative bacteria. In the lab, an additional 80 mL of tryptic soy broth was added to each Whirl-Pak containing a sponge used for the carcass swab. These sponges were then incubated at 25 °C for 2 hours followed by 42 °C for 6 hours. The sponges were then kept at 4 °C until the following day when they were plated onto MacConkey agar (3). If the samples plated on MacConkey agar grew colonies phenotypically consistent with *Escherichia coli*, one of the colonies was randomly selected and isolated for further testing. After isolation, the isolates were subjected to indole testing for confirmation of *E. coli.* Indole positive colonies were presumed to be *E. coli.*

The Kirby-Bauer disk diffusion assay was used to test for resistance to twelve different antimicrobials: ampicillin (10µg), tetracycline (30µg), neomycin (30µg), gentamicin (10µg), chloramphenicol (30µg), ceftiofur (30µg), nalidixic acid (30µg), sulfamethoxazole trimethoprim (23.75; 1.25 µg), cefoxitin (30µg), ciprofloxacin (5µg), ceftriaxone (30µg), and streptomycin

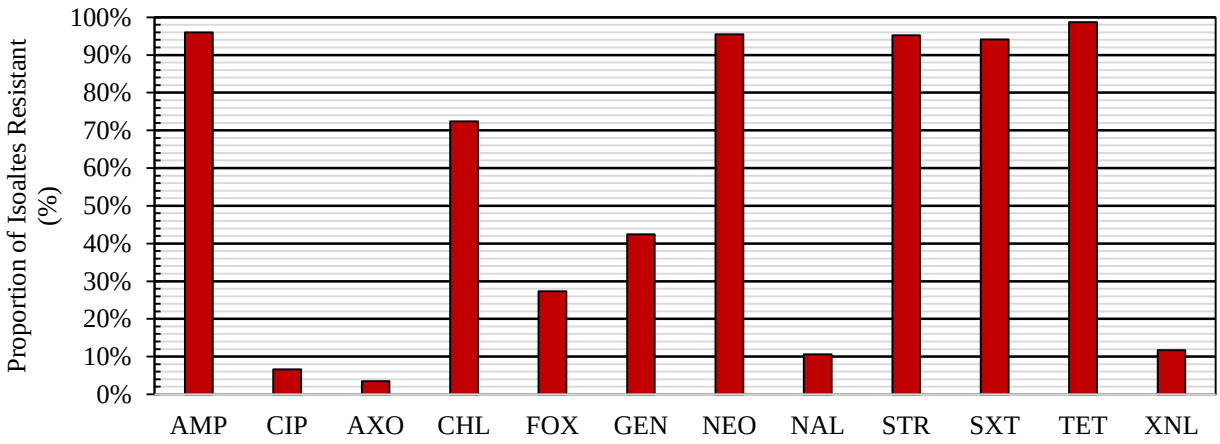
(10µg). To perform the assay, a colony from each isolate was used to inoculate a test tube of phosphate buffered saline to match the turbidity of a 0.5 McFarland standard. Turbidity was subjectively confirmed using a purchased standard and a Wickerham card. After subjective confirmation, the absorbance of the inoculations were read at 625 nm using a Thermo-Scientific SPECTRONIC 200 Visible Spectrophotometer to ensure consistency among tested samples; excepted absorbance readings were between 0.08-0.13. After obtaining the proper dilution, a sterile cotton swab was saturated with the inoculation and used to streak a lawn on a 150 mm plate of Mueller-Hinton agar. Following the inoculation of the media, the twelve antimicrobials were placed on agar using a dispenser. After incubating for 18-24 hours, each antimicrobials zone of inhibition was measured in millimeters for each sample. Samples were determined to be susceptible, intermediate, or resistant to each antimicrobial using breakpoints determined by the Clinical Laboratories Standard Institute (CLSI). Currently there are no CLSI breakpoints to analyze ceftiofur resistance in *E. coli* of bovine origin; therefore, the resistance breakpoints for bovine respiratory disease were used following the study conducted by Donaldson et al (4). ATCC control strains *E. coli* 29522, *Staphylococcus aureus* 25923, and *Pseudomonas aeruginosa* 27853 were used as controls.

To test the hypothesis that the prevalence of resistance in *E. coli* isolates changed through production stages, isolates recovered from farm fecal samples, harvest fecal samples, pre-evisceration swabs, and post-evisceration swabs were categorized as having reduced susceptibility or no reduced susceptibility. The isolate categorization for each antimicrobial was included as the response variable separate generalized linear mixed models. Farm and Time (production stage) were included as a fixed effects, and a random intercept was included for the calf. P-values less than 0.05 were considered statistically significant.

RESULTS

E. coli was recovered from all of the fecal samples collected on farm and fecal samples collected from viscera at harvest. When analyzing resistance among farm fecal samples, more than 98% (372/377) were resistant to two or more antimicrobials tested while only 0.5% (2/377) were pansusceptible to the antimicrobials tested. Resistance among the farm fecal isolates was greater than 50% for the following five antimicrobials: ampicillin, chloramphenicol, neomycin, streptomycin, sulfamethoxazole trimethoprim, and tetracycline (Figure 1).

Figure 1: Proportion of *E. coli* isolates collected on farms resistant to twelve antimicrobials



	AMP	CIP	AXO	CHL	FOX	GEN	NEO	NAL	STR	SXT	TET	XNL
# of Isolates Resistant	362	25	13	273	103	160	360	40	359	355	372	44
Total # of Isolates	377	377	377	377	377	377	377	377	377	377	377	377
Percentage	96.02%	6.63%	3.45%	72.41%	27.32%	42.44%	95.49%	10.61%	95.23%	94.16%	98.67%	11.67%

The Kirby-Bauer assay allowed for common resistance phenotypes to be determined (Table 1). These resistance phenotypes may indicate resistance genes carried by each isolate. In addition, resistance phenotypes may be compared with antimicrobial treatment records to identify associations with antimicrobial use. To determine if the farm isolates carry resistance genes with the potential to undergo horizontal gene transfer, additional genotypic work would be required.

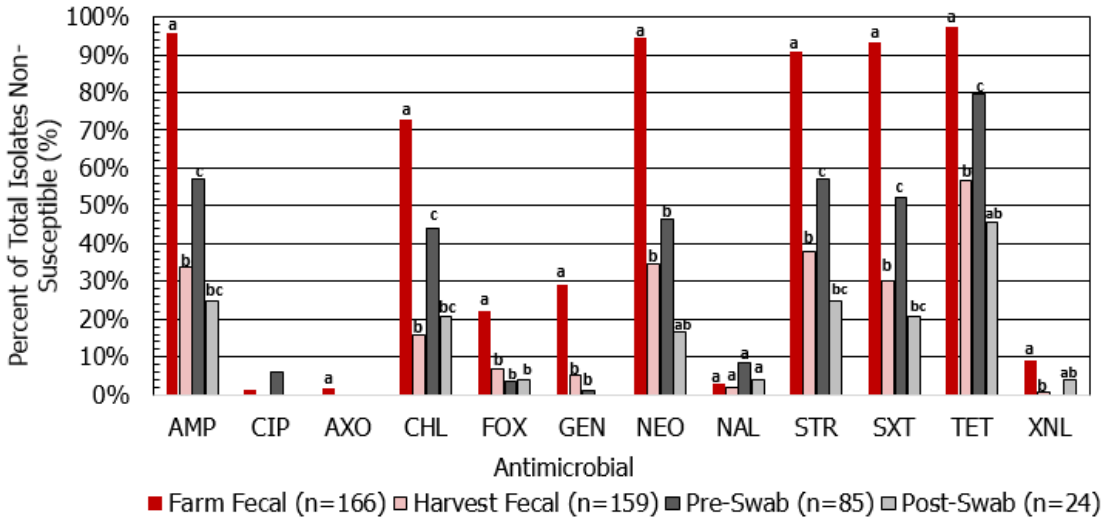
Table 1: Resistance Phenotypes among Farm Fecal Samples

Resistance Phenotypes of Farm Fecal Samples											
	Farm (Percentage of Isolates)										
Phenotype	1	2	3a	3b	4	5	6	7	8	Collective (%)	Total
AMP-CHL-NEO-STR-SXT-TET	28.6	16.7	31.7	33.3	43.9	16.7	-	7.0	23.8	22.28%	84
AMP-NEO-STR-SXT-TET	21.4	2.4	2.4	38.1	17.1	9.5	9.5	18.6	11.9	14.59%	55
AMP-CHL-GEN-NEO-STR-SXT-TET	4.8	-	56.1	14.3	4.9	14.3	16.7	4.7	2.4	13.00%	49
AMP-CHL-FOX-GEN-NEO-STR-SXT-TET	-	11.9	-	2.4	2.4	11.9	14.3	11.6	7.1	6.90%	26
AMP-CHL-FOX-NEO-STR-SXT-TET	2.4	28.6	-	-	-	4.8	4.8	2.3	2.4	5.04%	19
AMP-GEN-NEO-STR-SXT-TET	4.8	2.4	2.4	4.8	2.4	-	7.1	14.0	-	4.24%	16
AMP-CHL-FOX-GEN-NEO-STR-SXT-TET-XNL	-	11.9	-	-	-	9.5	2.4	4.7	2.4	3.45%	13
AMP-CHL-NEO-SXT-TET	4.8	-	-	-	9.8	4.8	-	2.3	-	2.39%	9
AMP-CIP-CHL-GEN-NEO-NAL-STR-SXT-TET	-	-	-	4.8	-	4.8	-	-	11.9	2.39%	9
AMP-CHL-FOX-NEO-STR-SXT-TET-XNL	-	7.1	2.4	-	-	2.4	-	-	2.4	1.59%	6
AMP-NEO-STR-TET	11.9	-	-	-	-	-	-	-	-	1.33%	5
AMP-CHL-GEN-NEO-NAL-STR-SXT-TET	2.4	2.4	-	-	-	2.4	-	-	4.8	1.33%	5
NEO--STR-SXT-TET	-	-	-	-	4.9	2.4	-	-	2.4	1.06%	4
AMP-CHL-NEO-STR-TET	-	-	-	-	-	-	-	9.3	-	1.06%	4
AMP-FOX-GEN-NEO-STR-SXT-TET	-	-	-	-	-	-	7.1	-	-	0.80%	3
AMP-FOX-GEN-NEO-STR-SXT-TET-XNL	-	4.8	-	-	-	-	-	2.3	-	0.80%	3
AMP-CHL-GEN-STR-SXT-TET	2.4	-	-	-	-	-	4.8	-	-	0.80%	3
AMP-CHL-FOX-GEN-NEO-STR-TET	-	-	-	-	-	-	-	7.0	-	0.80%	3
AMP-AXO-CHL-FOX-NEO-STR-SXT-TET-XNL	-	2.4	2.4	-	-	-	2.4	-	-	0.80%	3
AMP-CIP-CHL-FOX-GEN-NEO-NAL-STR-SXT-TET-XNL	-	-	-	-	-	4.8	2.4	-	-	0.80%	3
PANSUSCEPTIBLE	-	2.4	-	-	2.4	-	-	-	-	0.53%	2
AMP-STR-SXT-TET	2.4	-	-	-	-	-	2.4	-	-	0.53%	2
AMP-NEO-SXT-TET	2.4	-	-	-	2.4	-	-	-	-	0.53%	2
AMP-NEO-NAL-STR-SXT-TET	2.4	-	-	-	2.4	-	-	-	-	0.53%	2
AMP-FOX-NEO-STR-SXT-TET	-	-	-	-	2.4	-	-	2.3	-	0.53%	2
AMP-CHL-GEN-NEO-STR-TET	-	-	-	-	-	-	2.4	2.3	-	0.53%	2
AMP-CHL-GEN-NEO-NAL-STR-SXT-TET-XNL	-	-	-	-	-	-	-	-	4.8	0.53%	2
AMP-CHL-FOX-NEO-SXT-TET	4.8	-	-	-	-	-	-	-	-	0.53%	2
AMP-CHL-FOX-NEO-NAL-STR-SXT-TET	-	-	-	-	-	-	-	-	4.8	0.53%	2
AMP-CHL-FOX-GEN-STR-SXT-TET	-	-	-	-	-	-	4.8	-	-	0.53%	2
AMP-CHL-FOX-GEN-NEO-NAL-STR-SXT-TET	-	-	-	-	-	-	-	2.3	2.4	0.53%	2
AMP-CHL-FOX-GEN-NEO-NAL-STR-SXT-TET-XNL	-	-	-	-	-	2.4	-	-	2.4	0.53%	2
AMP-AXO-FOX-NEO-STR-SXT-TET-XNL	2.4	-	-	-	-	-	2.4	-	-	0.53%	2
AMP-AXO-CHL-FOX-GEN-NEO-STR-SXT-TET-XNL	-	-	-	-	-	2.4	2.4	-	-	0.53%	2
AMP-CIP-CHL-NEO-NAL-STR-SXT-TET	-	-	2.4	-	-	-	-	2.3	-	0.53%	2
AMP-CIP-CHL-FOX-GEN-NEO-NAL-STR-SXT-TET	-	-	-	-	-	-	2.4	2.3	-	0.53%	2
AMP-CIP-AXO-CHL-GEN-NEO-NAL-STR-SXT-TET-XNL	-	-	-	-	-	-	-	2.3	2.4	0.53%	2
AMP-CIP-AXO-CHL-FOX-GEN-NEO-NAL-STR-SXT-TET-XNL	-	-	-	-	-	-	4.8	-	-	0.53%	2
TET	2.4	-	-	-	-	-	-	-	-	0.27%	1
STR	-	2.4	-	-	-	-	-	-	-	0.27%	1
GEN-STR-SXT-TET	-	-	-	-	-	-	2.4	-	-	0.27%	1
CHL-GEN-NEO-STR-SXT-TET	-	-	-	-	-	2.4	-	-	-	0.27%	1
CIP	-	-	-	-	2.4	-	-	-	-	0.27%	1
CIP-CHL-GEN-NEO-NAL-STR-SXT-TET	-	-	-	-	-	-	-	-	2.4	0.27%	1
AMP-SXT-TET	-	-	-	-	2.4	-	-	-	-	0.27%	1
AMP-NEO-STR	-	-	-	-	-	-	-	2.3	-	0.27%	1
AMP-CHL-NEO-STR-SXT-TET-XNL	-	2.4	-	-	-	-	-	-	-	0.27%	1
AMP-CHL-NEO-NAL-STR-SXT-TET	-	-	-	-	-	-	-	-	2.4	0.27%	1
AMP-CHL-FOX-STR-TET	-	2.4	-	-	-	-	-	-	-	0.27%	1
AMP-AXO-CHL-GEN-NEO-NAL-STR-SXT-TET-XNL	-	-	-	-	-	-	-	-	2.4	0.27%	1
AMP-AXO-CHL-FOX-GEN-NEO-NAL-STR-SXT-TET-XNL	-	-	-	2.4	-	-	-	-	-	0.27%	1
AMP-CIP-CHL-NEO-STR-SXT-TET	-	-	-	-	-	-	-	-	2.4	0.27%	1
AMP-CIP-CHL-FOX-NEO-NAL-STR-SXT-TET	-	-	-	-	-	-	-	-	2.4	0.27%	1
AMP-CIP-CHL-FOX-GEN-NEO-STR-SXT-TET-XNL	-	-	-	-	-	2.4	-	-	-	0.27%	1
CHL-GEN-NEO-STR-TET	-	-	-	-	-	-	2.4	-	-	0.27%	1
Total Number of Isolates	**42**	**42**	**41**	**42**	**41**	**42**	**42**	**43**	**42**		**377**

For the longitudinal portion of the study, the calves sampled on farms 1, 2, 3a, and 4 were followed to harvest for additional sample collection. In regards to the fecal samples collected at harvest, 52% (73/159) of *E. coli* isolates were resistant to two or more antimicrobials while the remaining 38% were pansusceptible. When evaluating the collected carcass swabs, *E. coli* was isolated from 52% (84/161) and 16% (24/150) of pre-evisceration and post-evisceration swabs respectively. Among the pre-evisceration carcass swabs, 36% (56/161) of the samples had an isolate that was resistant to at least two of the antimicrobials tested while 17.85% were pansusceptible. Of the post-evisceration swabs, fewer isolates were resistant to more than one antimicrobial tested, 4.6% (7/150) while 41.67% were pansusceptible to the panel of twelve antimicrobials tested.

There was a general decrease in the prevalence of resistance between the fecal samples collected on farm and at harvest. For 9 of the 12 tested antimicrobials, this decrease was statistically significant. In contrary to the proposed hypothesis, there was a numerical increase in the proportion of isolates resistant between harvest fecal samples and pre-evisceration carcass swabs for 7 of the 12 antimicrobials tested, with five having a statistically significant increase. When comparing the prevalence of resistance among pre-evisceration swabs to post-evisceration swabs, there was a decrease in prevalence in nine of the antimicrobials but only tetracycline had a statistically significant decrease (Figure 2).

Figure 2: Proportion of *E. coli* Isolates Resistant from the Four Cohorts Sampled at Four Time Points and their Statistical Significance for each Antimicrobial



As previously mentioned, the Kirby-Bauer disk diffusion assay can be utilized to determine resistance phenotypes. Resistance phenotypes were determined for each isolate collected and the percentage of isolates for each phenotype was calculated. Table 2 shows common phenotypes found among all of the isolates and their prevalence at each sampling stage. Similar phenotypes among isolates collected at various time points may indicate transmission of the bacteria but would require genotypic work for confirmation.

Table 2: Common Resistance Phenotypes Found Among the Four Time Points Sampled

Common Resistance Phenotypes Among Sampling Time Points	Percentage of Isolates					
Phenotype	**Farm Fecal**	**Harvest Fecal**	**Pre-Evisceration**	**Post-Evisceration**	**Collective (%)**	**Total # Isolates**
AMP-CHL-NEO-STR-SXT-TET	22.3	7.5	21.4	12.5	18.2%	117
Pansusceptible	0.5	39.0	17.9	41.7	13.8%	89
AMP-NEO-STR-SXT-TET	14.6	8.2	4.8	-	11.2%	72
AMP-CHL-GEN-NEO-STR-SXT-TET	13.0	-	-	-	7.6%	49
TET	0.3	12.6	10.7	20.8	5.4%	35
AMP-CHL-FOX-GEN-NEO-STR-SXT-TET	6.9	-	-	-	4.0%	26
AMP-CHL-FOX-NEO-STR-SXT-TET	5.0	2.5	1.2	-	3.7%	24
AMP-GEN-NEO-STR-SXT-TET	4.2	3.1	1.2	-	3.4%	22
AMP-CHL-NEO-SXT-TET	2.4	1.9	2.4	-	2.2%	14
AMP-CHL-FOX-GEN-NEO-STR-SXT-TET-XNL	3.4	0.6	-	-	2.2%	14
AMP-CIP-CHL-GEN-NEO-NAL-STR-SXT-TET	2.4	-	-	-	1.4%	9
STR-TET	-	3.1	2.4	-	1.1%	7
AMP-TET	-	2.5	3.6	-	1.1%	7
AMP-NEO-STR-TET	1.3	0.6	1.2	-	1.1%	7
NEO-STR-TET	-	2.5	1.2	4.2	0.9%	6
AMP-CHL-FOX-NEO-STR-SXT-TET-XNL	1.6	-	-	-	0.9%	6
AMP-CHL-NEO-STR-TET	1.1	-	1.2	-	0.8%	5
AMP-CHL-GEN-NEO-NAL-STR-SXT-TET	1.3	-	-	-	0.8%	5
STR	0.3	1.3	1.2	-	0.6%	4
NEO-STR-SXT-TET	1.1	-	-	-	0.6%	4
AMP-STR-TET	-	1.3	2.4	-	0.6%	4
AMP-STR-SXT-TET	0.5	-	2.4	-	0.6%	4
AMP-NEO-NAL-STR-SXT-TET	0.5	-	2.4	-	0.6%	4
AMP-FOX-NEO-STR-SXT-TET	0.5	1.3	-	-	0.6%	4
AMP-CHL-STR-TET	-	0.6	2.4	4.2	0.6%	4
AMP-CIP-CHL-NEO-NAL-STR-SXT-TET	0.5	-	2.4	-	0.6%	4
NEO-TET	-	1.9	-	-	0.5%	3
NEO-SXT-TET	-	1.3	1.2	-	0.5%	3
CHL-STR-TET	-	0.6	2.4	-	0.5%	3
AMP-FOX-GEN-NEO-STR-SXT-TET	0.8	-	-	-	0.5%	3
AMP-FOX-GEN-NEO--STR-SXT-TET-XNL	0.8	-	-	-	0.5%	3
AMP-CHL-STR-SXT-TET	-	-	2.4	4.2	0.5%	3
AMP-CHL-GEN-STR-SXT-TET	0.8	-	-	-	0.5%	3
AMP-CHL-FOX-NEO-SXT-TET	0.5	-	1.2	-	0.5%	3
AMP-CHL-FOX-GEN-NEO-STR-TET	0.8	-	-	-	0.5%	3
AMP-AXO-CHL-FOX-NEO-STR-SXT-TET-XNL	0.8	-	-	-	0.5%	3
AMP-CIP-CHL-FOX-GEN-NEO-NAL-STR-SXT-TET-XNL	0.8	-	-	-	0.5%	3
NAL	-	0.6	-	4.2	0.3%	2
CHL-NEO-SXT-TET	-	-	2.4	-	0.3%	2
CHL-GEN-STR-SXT-TET	0.5	-	-	-	0.3%	2
AMP-NEO-SXT-TET	0.5	-	-	-	0.3%	2
AMP-FOX-STR-SXT-TET	-	1.3	-	-	0.3%	2
AMP-CHL-GEN-NEO-STR-TET	0.5	-	-	-	0.3%	2
AMP-CHL-GEN-NEO-NAL-STR-SXT-TET-XNL	0.5	-	-	-	0.3%	2
AMP-CHL-FOX-NEO-NAL-STR-SXT-TET	0.5	-	-	-	0.3%	2
AMP-CHL-FOX-GEN-STR-SXT-TET	0.5	-	-	-	0.3%	2
AMP-CHL-FOX-GEN-NEO-NAL-STR-SXT-TET	0.5	-	-	-	0.3%	2
AMP-CHL-FOX-GEN-NEO-NAL-STR-SXT-TET-XNL	0.5	-	-	-	0.3%	2
AMP-AXO-FOX-NEO-STR-SXT-TET-XNL	0.5	-	-	-	0.3%	2
AMP-AXO-CHL-FOX-GEN-NEO-STR-SXT-TET-XNL	0.5	-	-	-	0.3%	2
AMP-CIP-CHL-FOX-NEO-NAL-STR-SXT-TET	0.3	-	1.2	-	0.3%	2
AMP-CIP-CHL-FOX-GEN-NEO-NAL-STR-SXT-TET	0.5	-	-	-	0.3%	2
AMP-CIP-AXO-CHL-GEN-NEO-NAL-STR-SXT-TET-XNL	0.5	-	-	-	0.3%	2
AMP-CIP-AXO-CHL-FOX-GEN-NEO-NAL-STR-SXT-TET-XNL	0.5	-	-	-	0.3%	2
OTHER	3.7	5.7	7.1	8.3	4.8%	31
Total Number of Isolates	**377**	**159**	**84**	**24**		**644**

DISCUSSION

The objective of this study was to estimate the prevalence of antimicrobial resistance at various time points within a vertically integrated veal calf production system, and provide preliminary data for future research projects to reduce the prevalence of antimicrobial resistance. The hypothesis was that the prevalence of antimicrobial resistance would decrease between each time point with resistance being highest in fecal samples collected on farm and continuing to decrease as the calf moves throughout the production and harvest process. The expected results were that resistance would decrease between farm fecal samples, harvest fecal samples, pre-evisceration carcass swabs, and post-evisceration carcass swabs. Results obtained from the fecal samples collected during this study support current literature with prevalence of resistance being higher in younger animals and declining as the animal matures. This decrease believed to be associated with higher levels of antimicrobial use for preventative measures and growth promotion and the maturation of the microflora of the gastrointestinal tract (5).

Looking beyond the fecal samples and to the potential transmission of antimicrobial resistance into the food supply, carcass contamination is thought to occur from contact with the animal hide during the dehiding process. From the pre-evisceration carcass samples collected, *E. coli* was isolated from 52% (73/159) of the swabs supporting this belief. Of the isolates recovered, the prevalence of resistance was expected to be similar to or lower than the prevalence of the harvest fecal samples because of fecal contamination of the hide. Contrary to the proposed hypothesis, a statistically significant increase in the prevalence of resistance was seen between harvest fecal samples and pre-evisceration carcass swabs for 7 of the 12 antimicrobials tested. These results may indicate that the population of bacteria found on the hide

varies from the bacteria found within the gastrointestinal tract, with the prevalence of resistance being higher in the bacteria on the hide than within the gastrointestinal tract.

At the final sampling point, *E. coli* was recovered from 16% (24/150) of post-evisceration swabs with a general decrease in the prevalence of resistance between pre-evisceration and post-evisceration. Although a reduction in resistance was seen and the general trend observed supports the hypothesis, it was only statistically significant for tetracycline. This may be due to the lower number of isolates obtained from post-evisceration carcass swabs (n=24) compared to the pre-evisceration swabs (n=84) and the ability to deduce statistical significance.

CONCLUSION

The purpose of this study was to obtain observational data on the prevalence of antimicrobial resistance at various time points in a vertically integrated veal calf production system. The data reported is an overview of all of the farms and calf cohorts sampled and provides critical data to assess the transmission of antimicrobial resistance into the human food supply. Although a general decreasing trend was observed, the results from the post-evisceration carcass swabs show that resistant bacteria are making it into the food supply creating a public health concern. These bacteria have the potential to transfer resistance genes to pathogenic bacteria and create a problem with treating diseases which affect humans.

With the data collected from this study, future research studies should focus on reducing the prevalence of antimicrobial resistance in the veal calf production system. Studies should look at factors that influence the prevalence of resistance at the various time points sampled. Not only should ways of reducing the prevalence be examined, an emphasis should be placed on reducing the transmission into the human food supply.

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