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Effects of Restricted Antimicrobial Exposure on Antimicrobial Resistance in Fecal *Escherichia coli* from Feedlot Cattle

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Abstract

Objectives: The primary objective was to evaluate differences in antimicrobial resistance among enteric bacteria recovered from feedlot cattle that were being raised without exposure to antimicrobial drugs (AMDs) and those reared using conventional practices.

Materials: Forty pens of feedlot cattle (4557 total animals) that were being fed without AMD exposures were selected for enrollment as were 44 pens of cattle (4913 total animals) being fed for production of conventional beef products at the same feedlots. Fecal samples were collected from the floors of pens approximately biweekly through the middle of the feeding period and again prior to slaughter. Samples were cultured to recover nontype-specific *Escherichia coli* (NTSEC) and *Salmonella enterica*, and isolates were evaluated for susceptibility to a panel of AMDs.

Results: Cattle enrolled in the study did not differ between groups in entry weight or finish weight, but cattle with restricted AMD and hormone exposures were fed for an average of 50 days longer than conventionally reared cattle ($p < 0.001$). Resistance among NTSEC isolates was most common to tetracycline, streptomycin, and sulfamethoxazole, and there were slightly higher prevalence of resistance among NTSEC isolates recovered from conventionally reared cattle. Therapeutic AMD exposures did not have a detectable impact on the prevalence of resistance among NTSEC. Although there were detectable temporal trends through the feeding period for resistance to tetracycline, naladixic acid, chloramphenicol, and cephalothin, the direction of trends differed among drugs and these trends were not associated with study groups. *S. enterica* was recovered rarely (0.73%) but at similar prevalences from cattle with both rearing methods.

Conclusions: These findings suggest that conventional feedlot production methods (including parenteral and in-feed use of AMDs) do not predictably or uniformly increase the prevalence of antimicrobial resistance among fecal NTSEC when compared with rearing methods that restrict exposure to AMDs.

Introduction

In recent years, increased efforts by producers and government agencies to improve control of food safety hazards have coincided with heightened media coverage regarding the human health impacts of zoonotic microbial pathogens and antimicrobial resistance. Consumer demand and the availability of "organic" and "natural" food, including beef products, have increased during a similar time period, whether this is coincidental or in some way a response to increased media attention. Organic agriculture is likely one of the fastest growing sectors of U.S. agriculture, having experienced a growth of ~20% per year for the last 15 years (Oberholtzer *et al.*, 2005). In 1990, organic food retail sales in the United States were estimated to be $1 billion and reached $21.1 billion in 2008, which was ~3.5% of all U.S. retail food sales (Dimitri and Oberholtzer, 2009). Although organic meat, poultry, and fish represent a relatively small portion of these sales (about $600 million in 2008, of which about 26% was from organic beef), it is one of the fastest growing sectors of the organic

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market in the United States (Dimitri and Oberholtzer, 2009). The number of beef cattle raised with organic methods in the United States increased about 20% growth annually between 2000 and 2005, reaching ~100,000 per year in 2005 (Dimitri and Oberholtzer, 2009). The terms "organic" and "natural" are not interchangeable as they relate to food products sold in the United States. In compliance with United States Department of Agriculture (USDA) regulations, beef labeled as being "organic" must be certified as being obtained from animals that have access to pasture in all stages of production, are only fed grains and forage that meet certified organic standards, and are not exposed to any antimicrobial drugs (AMDs), hormone implants, and artificial anthelmintic compounds. Beef and other food products can be labeled as being "natural" if they are minimally processed and do not contain artificial or synthetic ingredients or coloring additives. Beef products can also be specifically labeled as coming from animals with no AMD or hormone exposures with proper certification (USDA Food Safety Inspection Service; www.fsis.usda.gov/Fact_Sheets/Meat_&_Poultry_Labeling_Terms/index.asp).

Research suggests that there are three major motivating factors related to consumers' decisions to purchase organic or natural products: perceived differences related to product safety, product quality, and ethical considerations regarding production methods (e.g., environmental considerations and animal welfare) (Hammitt, 1990; Grannis and Thilmany, 2000; Williams and Hammitt, 2000, 2001; Harper and Makatouni, 2002; Yiridoe *et al.*, 2005; Magkos *et al.*, 2006; Thilmany, 2006; Thilmany *et al.*, 2006). Studies have shown that consumers who purchase organic foods are willing to pay a premium for these products, in part because they believe they are more nutritious and less hazardous than the conventional products (Williams and Hammitt, 2000, 2001; Thilmany *et al.*, 2006). However, many believe there is insufficient scientific evidence to objectively support these claims (Magkos *et al.*, 2006). Specifically, there are no studies showing that meat sold in the United States as "organic" or "natural" products are more nutritious or safer relative to the presence of microbial pathogens or the presence of bacteria that are resistant to AMDs.

The majority of beef produced in the United States is derived from cattle that are purchased from a large number of small herds-of-origin as young animals and then aggregated in intensively managed feedlots where they are fed high-energy diets until they reach harvest weight. Exposure to AMDs is relatively infrequent prior to when cattle arrive at intensively managed backgrounding units and feedlots. However, cattle are commonly exposed to AMDs after placement in intensively managed production units for treatment of clinical disease, to prevent disease and mitigate subclinical bacterial infections, and to improve production efficiency (USDA, 1995, 2000). A wide variety of AMDs are licensed in the United States for treatment of sick cattle or for metaphylactic use, including florfenicol, cephalosporins, macrolides, fluoroquinolones, penicillins, sulfonamides, and tetracycline compounds. Other drugs such as tylosin and tetracycline compounds are commonly fed to reduce the occurrence and consequences of liver abscessation. Ionophores such as monensin are commonly fed for the sole purpose of improving growth efficiency (Potter *et al.*, 1985) as feeding these compounds alters the ecology of microflora in the rumen, thereby promoting more efficient metabolism. Ionophores are very poorly absorbed from the gastrointestinal (GI) tract and these drugs are never used in humans or other nonruminants because they are toxic to these species.

The primary purposes of this study were to investigate the potential associations between oral and parenteral AMD exposures and the occurrence of resistance among enteric bacteria recovered from feedlot cattle that are reared using "conventional" production methods and those reared without hormone implants and with no exposure to AMDs ("natural" production).

Materials and Methods

Study overview

A prospective longitudinal design was used for this investigation. Pens of cattle with no or minimal AMD exposures were enrolled for comparison with pens of cattle with typical AMD exposures. Fecal samples were collected from the floors of pens approximately biweekly through the middle of the feeding period (approximately days 0 to 70) and then prior to slaughter. All fecal samples were cultured to recover nontype-specific *Escherichia coli* (NTSEC) and *Salmonella enterica*, and isolates were evaluated for susceptibility to a standardized panel of AMDs. Comparisons of susceptibility results were made for isolates recovered from cattle raised using the two production schemes (natural vs. conventional). Additionally, temporal trends and associations between therapeutic AMD exposures and resistance were investigated. Study protocols were approved by the CSU Animal Care and Use Committee before initiation of investigations.

Feedlots and cattle

Cattle were purposefully selected and enrolled shortly after they arrived at three large commercial feedlots in Colorado (feedlots were not affiliated with each other). Most cattle (8480 cattle housed in 76 pens) were enrolled in the study at Feedlot A, which had a one-time capacity of ~18,000 cattle. A small number of cattle were also enrolled at two other feedlots: Feedlot B had a one-time of ~4000 animals (717 cattle managed in 5 pens), and Feedlot C had a capacity of ~35,000 animals (273 total cattle managed in 3 pens). Facilities and management procedures at these operations were typical of large beef feedlots located in the western United States and a variety of different types of cattle were fed year-round at these facilities.

Cattle enrolled in this study were domestic-source medium weight (~500–800 lb at entry), and the beef breeds (type) were heifers and steers. The two study groups of interest were animals raised for natural beef production and those raised using conventional methods. Cattle enrolled in the "natural" production group had not received AMDs, hormone implants, or anthelmintic drugs prior to arrival and were not exposed to these agents while at the feedlot. The exception to this was that after arrival at the feedlot, cattle that were diagnosed with bacterial infections were treated parenterally with AMDs and returned to their home pens. In general, when a pen of "natural" cattle was selected for enrollment, a pen of "conventional" cattle was enrolled at the same time based upon feedlot, similarity of cattle type, and arrival date at the feedlot.

After identification of production methods (conventional vs. natural), cattle were grouped in pens for management

within each feedlot according to owner, weight, and sex. Cattle being managed with restricted antimicrobial exposure were generally procured in large groups directly from their farms-of-origin, whereas conventionally reared cattle were generally procured in smaller groups from commercial auction markets. Thus, although not documented as part of this study, cattle enrolled in the conventional rearing group had a much larger number of farms-of-origin and generally more heterogeneous in terms of background exposures and experiences than were cattle enrolled in the natural production group. Assignment of specific housing locations (pens) for groups of cattle was made irrespective of the disease history and management strategies employed with cattle that had been previously housed in those same pens. Although cattle in the natural production group were sometimes clustered in adjacent pens to facilitate feeding, this was not strictly true for all cattle enrolled in the study. Thus, although not documented, cattle being managed with restricted AMD exposure could have been placed in or adjacent to pens that had previously been used with conventional rearing practices, and vice versa. Vaccines for respiratory and clostridial diseases, anthelmintics, and hormone implants were administered at the time when cattle entered the feedlots, and protocols were adjusted by the feedlot managers according to the intended production strategy (conventional vs. natural), the type of cattle, and the perceived risk of disease. AMDs were not administered prophylactically or metaphylactically at the time of initial processing to any cattle enrolled in this study. All cattle were fed primarily corn-based rations.

Trained feedlot personnel visually evaluated all cattle on a daily basis to identify animals that were clinically ill. Sick cattle were moved from their pen to a hospital facility where personnel worked under the direction of veterinarians to characterize their illness. Cattle thought to be clinically affected by bacterial infections were treated with therapeutic doses of AMDs in accordance with treatment protocols prescribed by supervising veterinarians. Treated animals were housed in hospital facilities until they recovered, at which time they were returned to their pen of origin. The most typical hospitalization period was 3 days. Records regarding parenteral treatment with AMDs were only available from the feedlot that supplied the majority of cattle enrolled in the study (Feedlot A). These records were downloaded from computerized databases maintained at the feedlot and were summarized by pen.

For Feedlot A, cattle diagnosed with respiratory disease were initially treated with enrofloxacin and those requiring additional treatments received florfenicol. When cattle that had been enrolled in the natural production group became ill, they were treated with AMDs using the same treatment protocols assigned to the conventionally reared cattle. These treated cattle were visually identified as no longer being eligible for use in natural beef products but were returned to their original pens and thus shared their housing environment with the remaining cohort of "natural" cattle until slaughter. In addition to treatment of sick cattle as described, cattle enrolled in the conventional rearing group were fed rations containing tylosin (a macrolide) and monensin (an ionophore) throughout the feeding periods in accordance with the U.S. Food and Drug Administration (FDA) regulations for in-feed medications. No cattle were treated with tetracycline class drugs by any route. Mandatory withdrawal were followed for all cattle treated with AMDs (Database of Approved Animal Drugs; www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts).

Sample collection

Investigators visited feedlots approximately biweekly to collect fecal samples from the floors of pens. Samples were collected from each pen at six targeted sampling dates: ~0, 15, 30, 45, and 60 days on feed (DOF) and within 30 days of slaughter. When pens of cattle were retained in the feedlot longer than anticipated after the initial preslaughter sample was collected (i.e., longer than 30 days), an additional preslaughter sample was collected. Sampling was more frequent during the first half of the feeding period because most infectious illness is identified and treated during this period in this class of feedlot cattle. Twenty fecal samples were collected at each sampling date. Investigators donned new disposable plastic boots and disposable exam gloves prior to entering each pen, and recently voided feces were arbitrarily selected for sampling by walking in a serpentine pattern across the pens. A sterile wooden tongue depressor was used to collect samples from each fecal pat; ~4 g of feces was collected from the center of the pat and placed into a sterile 50-mL tube. A new tongue depressor was used to collect each sample. Samples were placed in a cooler with ice packs and transported to the laboratory within 12 hours of collection.

Culture and isolation of NTSEC

A cotton swab was used to plate samples on MacConkey agar, and the plates were streaked for isolation and then incubated for 18–24 hours at 35°C. Isolates that fermented lactose and had appropriate morphology were subcultured on tryptic soy agar with 5% sheep blood and then tested for indole reaction. A presumptive identification of NTSEC was based on colony morphology, lactose fermentation, and positive indole reaction. A single isolate was selected from each plate (i.e., one per fecal sample) and archived for susceptibility testing by freezing at −80°C.

Culture and isolation of S. enterica

Tetrathionate broth with iodine (40 mL; Difco, Sparks, MD) was added to sterile tubes containing ~4 g of feces and incubated at 42°C for 24 hours. After incubation, samples were vortexed, and 100 μL of tetrathionate broth was added to 9 mL Rappaport R-10 medium (Difco) and incubated at 35°C for 24 hours, then streaked to Xylose-Lysine-Tergitol 4 agar (Hardy Diagnostics, Santa Maria, CA), incubated at 35°C for 24 hours, and rechecked at 48 hours. Colonies that exhibited growth characteristics consistent with *S. enterica* were subcultured to tryptic soy agar with 5% sheep blood, incubated at 35°C for 24 hours, and then evaluated for agglutination using poly-O grouping anti-sera (Hardy Diagnostics). Isolates that demonstrated agglutination were assigned a presumptive identification of *Salmonella*, and a presumptive serogroup designation was assigned using agglutination tests with commercial group-specific anti-sera (Hardy Diagnostics). A single isolate was selected from each plate and archived for serotyping and susceptibility testing at a later time. All *Salmonella* isolates were submitted to U.S. Department of

Agriculture–National Veterinary Services Laboratories (Ames, IA) for serovar classification.

Antimicrobial susceptibility testing

Our initial intent was to test the susceptibility of all isolates collected. However, because of resource limitations that developed during the course of the investigation, it was necessary to select a subset of the archived NTSEC isolates for susceptibility testing. Complete sets of isolates collected from a pen on a given date (i.e., all 20 samples collected on a specific date) were tested, and at least three sets of isolates were analyzed for susceptibility from each of the enrolled pens (Table 1). This included the first set collected, one set collected from the middle of the feeding period, and the set collected at the end of the feeding periods. Other sets of isolates were purposefully selected to represent a broad cross-section of pens and sampling periods (DOF). Sample sets were selected for testing without knowledge of study group (natural or conventional cattle), treatment histories, *Salmonella* recovery rate, or information about susceptibility of NTSEC isolates collected at other time points.

Minimum inhibitory concentrations were evaluated for a standardized panel of 16 AMDs (NARMS CMV7CNCD; Trek Diagnostics, Westlake, OH) by use of a semiautomated broth microdilution system (Sensititre; Trek Diagnostics) in accordance with the manufacturer's instructions and guidelines published by the Clinical and Laboratory Standards Institute (CLSI, 2006). Reference strains of *E. coli* (American Type Culture Collection [ATCC] 25922), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 29213), and *Pseudomonas aeruginosa* (ATCC 27853) were used as reference isolates for quality control (all from American Type Culture Collection, Manassas, VA). Isolates were categorized as susceptible, intermediate, or resistant to AMDs using interpretive guidelines published by Clinical and Laboratory Standards Institute (CLSI, 2006). Interpretive criteria used by the National Antimicrobial Resistance Monitoring System for Gram-negative isolates were used to guide classification when specific drug–bacteria criteria were not available from CLSI (Table 2; www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM182918.pdf).

Table 1. Summary of Fecal Nontype-Specific *Escherichia coli* Isolates That Were Evaluated for Susceptibility to Antimicrobial Drugs

Category	*Group*	*Pens*	*Samples*
All samples	Total	84	8882
Sampling dates evaluated per pen	3 Dates	1	60
	4 Dates	3	239
	5 Dates	55	5376
	6 Dates	8	949
	7 Dates	14	2258
Weeks on feed when sampled	1–3 weeks	74	2295
	4–6 weeks	61	1458
	7–9 weeks	41	1098
	10–12 weeks	21	420
	13–16-weeks	83	2612
	17–27 weeks	48	999

Isolates were obtained from all pens on multiple dates (pen, $n = 84$; individual pen sampling dates, $n = 328$).

Data analysis

Data regarding signalment of cattle enrolled in the study, parenteral and in-feed administration of AMDs, vaccines, implants, mortality, and antimicrobial susceptibility results were entered into a computer database and validated to ensure data integrity. Data regarding cattle characteristics, treatments, morbidity, and mortality were summarized at the pen level for statistical comparisons. Parenteral exposures to AMDs were summarized as the total of defined daily doses (DDD) of AMDs used during the feeding period for each pen of cattle and as the number of DDDs per 100 heads of cattle in pens. A DDD was defined as number of days of exposure to therapeutic concentrations of drug based on an assumed average maintenance dosage of a specific drug. Each dose of enrofloxacin and florfenicol administered was considered to have 3 days of therapeutic exposure, which is consistent with FDA-approved label claims for these drugs (Database of Approved Animal Drugs; www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts). As such, each treatment was assumed to contribute three DDDs.

Distributions of values for minimum inhibitory concentrations and susceptibility classification were summarized and evaluated descriptively. Resistance classifications were used to create phenotypic profiles for isolates, which were summarized and evaluated descriptively. Poisson regression was used to obtain estimates of the resistance prevalence and 95% confidence intervals (CIs) for each study group. Regression analysis using generalized estimating equation methods was used to correct prevalence estimates for lack of independence associated with collecting multiple fecal samples within each pen of cattle on a particular date and by repeated sampling of pens of cattle over time (SAS PROC GENMOD v9.2; SAS Institute, Cary, NC). Correlation structures nested each unique sample set within each unique group (pen) of cattle. Production method (rearing for production of conventional or natural beef) was used as the exposure variable of interest for these analyses, and separate models were developed to estimate resistance prevalences for 15 of the 16 AMDs evaluated. It was not possible to estimate the prevalence of isolates resistant to amikacin, as the range of dilutions included on the commercial plate did not include the resistance breakpoint for that drug (16 μg/mL). Logistic regression was used to analyze whether isolates recovered from the two groups of cattle differed in their likelihood of being resistant to $\geq$1 AMD versus being pansusceptible, being resistant to >2 AMDs versus being resistant to $\leq$2 drugs, and being resistant to >3 AMDs versus being resistant to $\leq$3 drugs (SAS PROC GENMOD v 9.2; SAS Institute). To facilitate analysis of temporal information, samples were grouped into six categories according to the DOF for the pen at the time of sample collection (1–3 weeks on feed, 4–6 weeks, 7–9 weeks, 10–12 weeks, 13–15 weeks, and 17–27 weeks). Group×time interactions were also evaluated when the analyses suggested that resistance prevalence varied over time for a particular drug. Resistance prevalences among isolates were compared at the first sampling period (1–3 weeks on feed) between groups to evaluate whether baseline prevalences were equivalent. To evaluate potential associations between parenteral treatment

Table 2. Minimum Inhibitory Concentrations for Nontype-Specific *Escherichia coli* Isolates Recovered from Feedlot Cattle

		Minimum inhibitory concentrations (μg/mL)[a]																
Drug	*Group*	*0.015*	*0.03*	*0.06*	*0.12*	*0.25*	*0.5*	*1*	*2*	*4*	*8*	*16*	*32*	*64*	*128*	*256*	*512*	*1024*
Amikacin	Natural						0.7% (31)	19.4% (809)	60.0% (2501)	17.6% (735)	2.2% (93)							
	Conventional						0.3% (15)	18.4% (867)	61.5% (2898)	17.5% (825)	2.3% (108)							
Amoxicillin–clavulanate	Natural							4.4% (183)	28.1% (1170)	61.8% (2576)	5.5% (230)	0.0% (2)	0.2% (7)	0.0% (1)				
	Conventional							5.0% (236)	29.5% (1392)	58.1% (2737)	7.2% (337)	0.1% (4)	0.1% (4)	0.1% (3)				
Ampicillin	Natural							5.6% (234)	32.8% (1368)	50.7% (2115)	8.3% (347)	0.9% (36)	0.1% (5)	1.5% (64)				
	Conventional							6.4% (300)	34.3% (1615)	47.2% (2223)	8.9% (420)	1.1% (51)	0.1% (6)	2.1% (98)				
Cefoxitin	Natural						0.0% (1)	1% (42)	26.5% (1105)	53.7% (2239)	16.3% (679)	2.3% (97)	0.1% (6)	0.0% (1)				
	Conventional						0.1% (6)	1.8% (87)	26.6% (1252)	53.6% (2525)	15.9% (749)	1.8% (87)	0.1% (7)	0.1% (6)				
Ceftiofur	Natural				10.9% (453)	65.8% (2743)	22.6% (944)	0.6% (24)	0% (0)	0.0% (1)	0.1% (3)	0.0% (1)						
	Conventional				12.9% (607)	62.2% (2933)	24.3% (1147)	0.4% (19)	0.0% (2)	0% (0)	0.0% (1)	0.1% (4)						
Ceftriaxone	Natural					99.6% (4154)	0.2% (7)	0.0% (2)	0.1% (4)	0% (0)	0.0% (1)	0.0% (1)	0% (0)	0% (0)	0% (0)			
	Conventional					99.6% (4692)	0.2% (8)	0.1% (4)	0.1% (4)	0.0% (1)	0.0% (1)	0.1% (3)	0% (0)	0% (0)	0% (0)			
Cephalothin	Natural								1.8% (74)	16.3% (679)	53.3% (2222)	23.9% (995)	4.5% (186)	0.3% (13)				
	Conventional								2.9% (135)	17.3% (816)	51.0% (2404)	23.9% (1127)	4.6% (217)	0.3% (14)				
Chloramphenicol	Natural								3.2% (133)	41.9% (1746)	51.0% (2128)	3.1% (131)	0% (0)	0.7% (31)				
	Conventional								3.4% (159)	42.4% (1998)	49.2% (2321)	3.6% (171)	0.0% (2)	1.3% (62)				
Ciprofloxacin	Natural	97.6% (4070)	1.3% (55)	0.3% (12)	0.2% (10)	0.3% (12)	0.1% (3)	0.0% (1)	0% (0)	0.0% (1)	0.1% (5)							
	Conventional	98.0% (4617)	0.9% (44)	0.1% (5)	0.3% (14)	0.4% (18)	0.1% (5)	0.1% (3)	0% (0)	0% (0)	0.1% (7)							
Gentamicin	Natural					30.5% (1270)	62.9% (2623)	2.4% (102)	0.0% (2)	0.0% (1)	0.1% (3)	0.0% (1)	0% (0)					
	Conventional					31.1% (1468)	61.6% (2902)	2.3% (110)	0.2% (11)	0.0% (0)	0.0% (2)	0.0% (1)	0% (0)					
Kanamycin	Natural										97.9% (4083)	1.4% (59)	0% (0)	0.1% (4)	0.6% (23)			
	Conventional										97.9% (4616)	1.2% (57)	0.0% (1)	0% (0)	0.8% (39)			
Naladixic acid	Natural						0% (0)	2.3% (96)	53.2% (2216)	41.6% (1736)	1.8% (76)	0.2% (9)	0.1% (5)	0.7% (31)				
	Conventional						0.0% (1)	2.2% (104)	54.8% (2582)	40.6% (1915)	1.2% (56)	0.2% (9)	0.1% (4)	0.9% (42)				
Streptomycin	Natural												88.7% (3699)	7.0% (291)	4.3% (179)			
	Conventional												85.8% (4044)	7.3% (344)	6.9% (325)			
Sulfamethoxazole	Natural											89.4% (3728)	0.5% (21)	0.0% (0)	0.0% (2)	0.0% (2)	0.2% (7)	9.8% (409)
	Conventional											85.9% (4049)	0.6% (30)	0.1% (4)	0.1% (6)	0.2% (9)	0.2% (11)	12.8% (604)
Tetracycline	Natural									50.5% (2106)	15.0% (627)	7.2% (302)	6.0% (250)	21.2% (884)				
	Conventional									38% (1790)	18.5% (871)	9.8% (462)	7.2% (337)	26.6% (1253)				
Trimethoprm–sulfamethoxazole	Natural				88.6% (3692)	7.8% (325)	3.0% (126)	0.2% (10)	0.0% (2)	0.0% (1)	0.3% (13)							
	Conventional				86.8% (4093)	9.0% (424)	3.7% (173)	0.1% (7)	0% (0)	0% (0)	0.3% (16)							

[a]The upper (right-most) line in the MIC range represents breakpoints for resistance. When present, the lower (left-most) line represents breakpoints for susceptibility. $n = 4169$ for isolates recovered from cattle raised without antimicrobial exposures; $n = 4713$ for isolates recovered from cattle raised with conventional methods. Values for amoxicillin–clavulanate refer to amoxicillin concentrations (clavulanate was included in wells at half of the amoxicillin concentration). Values for trimethoprim–sulfamethoxazole represent trimethoprim concentrations (sulfamethoxazole was included in wells at 19 times the concentration of trimethoprim).

Table 3. Adjusted Percentage of Resistance Among Nontype-Specific *Escherichia coli* Isolates Controlling for Effects of Clustering and for Days on Feed at the Time of Sampling

	Natural cattle		*Conventional cattle*		
Antimicrobial drug	*Estimate*	*95% CI*	*Estimate*	*95% CI*	p-*Value*
Amoxicillin–clavulanate	0.2%	(0.1%–0.6%)	0.2%	(0.1%–0.3%)	0.68
Ampicillin	1.7%	(1.2%–2.4%)	2.2%	(1.7%–2.8%)	0.12
Cefoxitin	0.2%	(0.1%–0.4%)	0.2%	(0.1%–0.3%)	0.98
Ceftiofur	0.1%	(0.04%–0.4%)	0.1%	(0.1%–0.3%)	0.91
Ceftriaxone	0%[a]		0%[a]		
Cephalothin	4.8%	(3.7%–6.1%)	4.9%	(4.2%–5.7%)	0.83
Chloramphenicol	0.7%	(0.5%–1.2%)	1.4%	(1.0%–1.9%)	0.02
Ciprofloxacin	0.2%	(0.05%–0.5%)	0.2%	(0.1%–0.4%)	0.98
Gentamicin	0.1%	(0.05%–0.4%)	0.1%	(0.04%–0.3%)	0.58
Kanamycin	0.7%	(0.3%–1.2%)	0.8%	(0.5%–1.3%)	0.46
Naladixic acid	0.9%	(0.5%–1.4%)	1.0%	(0.7%–1.4%)	0.64
Streptomycin	11.3%	(9.7%–13.1%)	14.2%	(12.9%–15.6%)	0.003
Sulfamethoxazole	10.0%	(8.4%–11.9%)	13.1%	(11.8%–14.5%)	0.002
Tetracycline	34.5%	(31.2%–38.1%)	43.5%	(40.9%–46.4%)	<0.0001
Trimethoprim–sulfamethoxazole	0.3%	(0.2%–0.8%)	0.3%	(0.2%–0.6%)	0.97

p-Values relate to differences in prevalence between the two study groups.
[a]No isolates were resistant to ceftriaxone.
95% CI, 95% confidence interval.

of cattle with AMDs and antimicrobial resistance in isolates, treatment rates were summarized for pens of cattle enrolled at Feedlot A; data from cattle enrolled at Feedlots B and C were not included in this analysis as treatment data were not provided by the feedlots. Separate models were used to analyze potential associations between resistance prevalence and enrofloxacin DDDs, florfenicol DDDs, and total DDDs. Separate models were also used to assess potential associations with DDDs accumulated per 100 heads of cattle in pens. A critical alpha of 0.05 was used for all statistical analyses.

Results

A total of 84 pens were enrolled in the study, of which 40 pens of cattle were raised using natural production ($n = 4557$ cattle) and 44 pens of cattle were raised using conventional methods ($n = 4913$ cattle). The mean arrival weight for pens of cattle was 727 lbs (pen-level standard deviation [SD] = 104 lbs), and the mean finishing weights for pens of cattle was 1208 lbs (pen-level SD = 80 lbs). There were no detectable differences between study groups in average arrival or finishing weights for pens ($p = 0.72$ and $p = 0.13$, respectively). However, there was a very large difference in average duration of feeding. The average DOF for pens of conventional cattle and for pens of natural cattle were 162 days (SD = 36 days) and 212 days (SD = 54 days), respectively ($p < 0.001$).

A total of 12,760 fecal samples were collected and cultured for recovery of NTSEC and *Salmonella* isolates. An isolate of NTSEC was recovered from every fecal sample. Antimicrobial susceptibility of 8882 NTSEC isolates collected from 328 pen sampling dates was evaluated (Tables 1 and 2).

Susceptibility among NTSEC isolates

Adjusting for potential correlation created by repeated sampling within pens on a given date and over time, the prevalence of resistance among all NTSEC isolates was greatest to tetracycline (39.3%), streptomycin (12.8%), sulfamethoxazole (11.6%), and cephalothin (4.8%; Tables 2 and 3). Differences in susceptibility were not statistically detectable among feedlots, and thus isolates recovered from different feedlots were pooled for these analyses. Susceptibilities to AMDs were generally very similar for isolates recovered from cattle raised using natural versus conventional production methods (Tables 2 and 3). Although differences in resistance prevalence were generally small to moderate between production groups, there were statistically detectable differences for chloramphenicol, streptomycin, sulfamethoxazole, and

Table 4. Number of Antimicrobial Drugs to Which Nontype-Specific *Escherichia coli* Isolates Were Resistant

Resistance number	*Natural cattle*	*Conventional cattle*	*Total*
Pan-susceptible	61.2% (2552)	52.5% (2473)	56.6% (5025)
1	23.1% (964)	26.8% (1264)	25.1% (2228)
2	7.3% (304)	10.2% (482)	8.8% (786)
3	7.1% (297)	8.8% (416)	8.0% (713)
4	0.9% (37)	0.9% (44)	0.9% (81)
5	0.1% (3)	0.3% (14)	0.2% (17)
6	0.05% (2)	0.1% (6)	0.1% (8)
7	0.05% (2)	0.2% (8)	0.1% (10)
8	0.02% (1)	0.1% (3)	0.05% (4)
9	0.1% (4)	0.04% (2)	0.1% (6)
10	0.1% (3)	0.02% (1)	0.05% (4)
11	0	0	0
12	0	0	0
13	0	0	0
14	0	0	0
15	0	0	0
16	0	0	0
Total isolates	4169	4173	8882

Estimates were not adjusted for effects of repeated sampling within pens of cattle.

Table 5. Resistance Phenotypes Among Nontype-Specific *Escherichia coli* Isolates

Study group	Frequency	Pct of Isolates from group	Resistance number	Amoxicillin–clavulanate	Ampicillin	Cefoxitin	Ceftiofur	Ceftriaxone	Cephalothin	Chloramphenicol	Ciprofloxacin	Gentamicin	Kanamycin	Naladixic acid	Streptomycin	Sulfamethoxazole	Tetracycline	Trimethoprim–sulfamethoxazole
Natural cattle	2552	61.2%	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	796	19.1%	1	–	–	–	–	–	–	–	–	–	–	–	–	–	R	–
	244	5.9%	3	–	–	–	–	–	–	–	–	–	–	–	R	R	R	–
	140	3.4%	1	–	–	–	–	–	R	–	–	–	–	–	–	–	–	–
	131	3.1%	2	–	–	–	–	–	–	–	–	–	–	–	R	–	R	–
	106	2.5%	2	–	–	–	–	–	–	–	–	–	–	–	–	R	R	–
	31	0.7%	2	–	–	–	–	–	R	–	–	–	–	–	–	–	R	–
	16	0.4%	3	–	R	–	–	–	–	–	–	–	–	–	R	–	R	–
	16	0.4%	3	–	–	–	–	–	–	–	–	–	R	–	R	–	R	–
	13	0.3%	2	–	R	–	–	–	–	–	–	–	–	–	–	–	R	–
	13	0.3%	1	–	–	–	–	–	–	–	–	–	–	R	–	–	–	–
	10	0.2%	2	–	–	–	–	–	–	R	–	–	–	–	–	–	R	–
	9	0.2%	4	–	R	–	–	–	–	–	–	–	–	–	R	R	R	–
	7	0.2%	3	–	–	–	–	–	R	–	–	–	–	–	R	–	R	–
	7	0.2%	4	–	–	–	–	–	–	R	–	–	–	–	R	R	R	–
	7	0.2%	1	–	–	–	–	–	–	–	–	–	–	–	R	–	–	–
	71	3.2%	Other phenotypes							Other phenotypes							Other phenotypes	
Conventional cattle	2473	52.5%	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	1105	23.4%	1	–	–	–	–	–	–	–	–	–	–	–	–	–	R	–
	309	6.6%	3	–	–	–	–	–	–	–	–	–	–	–	R	R	R	–
	207	4.4%	2	–	–	–	–	–	–	–	–	–	–	–	R	–	R	–
	183	3.9%	2	–	–	–	–	–	–	–	–	–	–	–	–	R	R	–
	124	2.6%	1	–	–	–	–	–	R	–	–	–	–	–	–	–	–	–
	51	1.1%	2	–	–	–	–	–	R	–	–	–	–	–	–	–	R	–
	31	0.7%	3	–	R	–	–	–	–	–	–	–	–	–	R	–	R	–
	31	0.7%	4	–	–	–	–	–	–	R	–	–	–	–	R	R	R	–
	18	0.4%	3	–	–	–	–	–	R	–	–	–	–	–	R	–	R	–
	18	0.4%	3	–	–	–	–	–	–	–	–	–	R	–	R	–	R	–
	17	0.4%	2	–	R	–	–	–	–	–	–	–	–	–	–	–	R	–
	12	0.3%	3	–	R	–	–	–	R	–	–	–	–	–	–	R	–	–
	12	0.3%	1	–	–	–	–	–	–	–	–	–	–	R	–	–	–	–
	11	0.2%	1	–	–	–	–	–	–	–	–	–	–	–	–	R	–	–
	10	0.2%	1	–	–	–	–	–	–	–	–	–	–	–	R	–	–	–
	101	4.1%	Other phenotypes							Other phenotypes							Other phenotypes	

Estimates were not adjusted for effects of repeated sampling within pens of cattle. Resistance number = number of drugs to which isolates were resistant.
R, resistant.

tetracycline (Table 3). However, resistance was also greater to these AMDs at the time when they were first sampled (1–3 weeks on feed). These differences were statistically greater for chloramphenicol and tetracycline ($p = 0.03$); resistance to chloramphenicol was found in 2.2% NTSEC recovered from conventionally reared cattle (95% CI = 1.4%–3.6%) versus 0.9% (0.3%–3.0%) isolates recovered from natural cattle ($p = 0.03$), and resistance to tetracycline was found in 36.7% NTSEC recovered from conventional cattle (95% CI = 32.1%–42.0%) versus 28.6% isolates from natural cattle (20.3%–40.5%; $p = 0.03$). Differences were numerically greater, but not statistically significant for sulfamethoxazole and streptomycin ($p > 0.05$).

Approximately half (56.6%) of NTSEC isolates were susceptible to all AMDs and 98.5% of isolates were resistant to ≤ 3 AMDs (Table 4). Isolates of NTSEC recovered from conventional cattle were more likely to be resistant to at least one AMD than were isolates recovered from natural cattle (47.5% vs. 38.8%, respectively; $p < 0.001$). Isolates from conventional cattle were also more likely to be resistant to ≥ 2 AMDs than were those from natural cattle (10.5% vs. 8.4%, respectively; $p = 0.01$). However, there was not a detectable difference between groups in the likelihood of being resistant to ≥ 3 AMDs ($p = 0.17$). Examination of resistance phenotypes for NTSEC isolates shows that these differences are mostly attributable to the differences in resistance to streptomycin, sulfamethoxazole, and tetracycline (Table 5).

Associations with parenteral AMD exposures

For the 76 pens that had therapeutic treatment data available, the number of AMD DDDs associated with parenteral treatments accumulated by pens of cattle varied dramatically. The total number of enrofloxacin DDDs accumulated across all cattle was 1296 (median per 100 heads per pen = 6.9, Q1 = 3.6, Q3 = 16.8), the total number of florfenicol DDDs accumulated among all cattle was 336 (median per pen = 0, Q1 = 0, Q3 = 3.7 per 100 heads per pen), and the sum of all AMDs accumulated in cattle was 1632 (median per 100 heads per pen = 8.0, Q1 = 3.8, Q3 = 18.2). There were no statistically detectable differences between study groups in total DDDs accumulated for enrofloxacin or florfenicol, but the median number of all DDDs accumulated per pen was greater for natural cattle even though the sum for the group was lower; the sum of all DDDs for natural cattle was 591 (median DDDs per 100 heads per pen = 9.3, Q1 = 4.8, Q3 = 17.0) compared with the sum of all DDDs for conventional cattle, which was 1041 (median per 100 heads per pen = 6.6, Q1 = 2.4, Q3 = 28.9; $p = 0.02$). Controlling for study group, there were no detectable associations between any of the accumulated DDD measurements and the prevalence of resistance. Most notably, there were no detectable associations between enrofloxacin exposures and resistance to naladixic acid or ciprofloxacin, and there was no detectable association between florfenicol exposures and resistance to chloramphenicol.

Temporal changes in resistance among NTSEC isolates

Controlling for rearing method (natural or conventional beef production), there were detectable differences in resistance prevalence across DOF categories for 4 of the 16 AMDs evaluated: cephalothin, chloramphenicol, naladixic acid, and tetracycline ($p < 0.05$; Fig. 1). For cephalothin, resistance

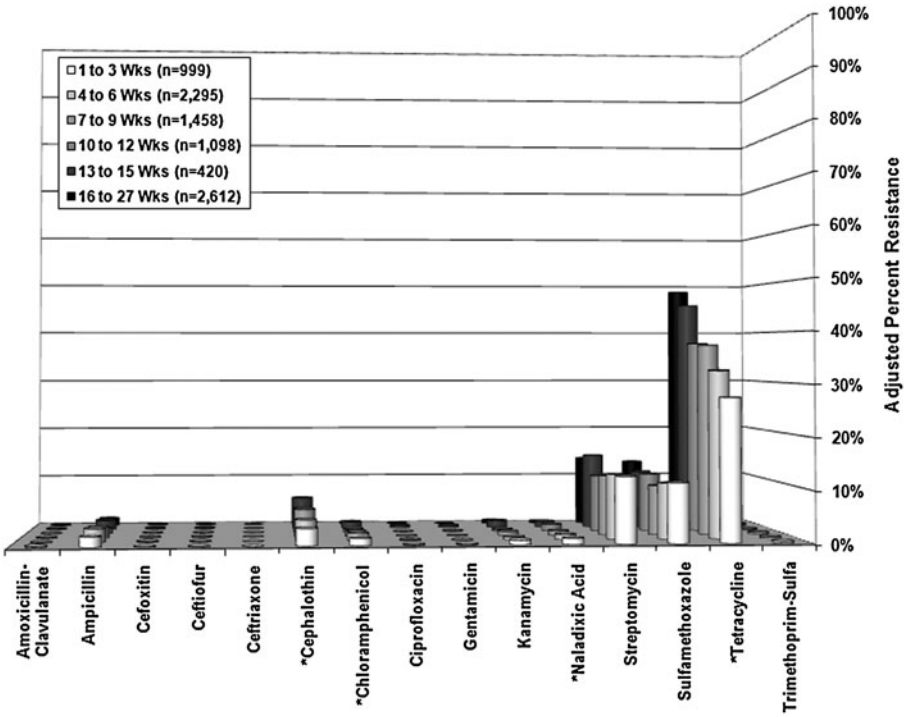

FIG. 1. Adjusted percentage of resistance among nontype-specific *Escherichia coli* isolates adjusted for differences between study groups and the effects of clustering. *Differences in resistance prevalence among sampling periods were statistically significant ($p < 0.05$).

prevalence was lower in samples collected during the first 6 weeks of the feeding period than it was later; resistance in weeks 7–12 was intermediate, and resistance was greatest during weeks 13–28 ($p < 0.05$). The least squares (LS) mean resistance prevalence for cephalothin, controlling for method of rearing, was 3.3% (95% CI = 2.3%–4.7%) in samples collected in weeks 1–3 compared with 5.5% (95% CI = 4.4%–7.0%) in samples collected in weeks 17–28 ($p = 0.02$). Resistance prevalence for tetracycline showed a similar pattern over the feeding period in comparison to cephalothin (lowest early, intermediate during the middle of the feeding period, and highest at the end; $p < 0.05$). The LS mean prevalence for resistance to tetracycline during the first 3 weeks on feed, controlling for differences related to rearing method, was 27.5% (95% CI = 22.8%–33.2%) during weeks 1–3 compared with 48.6% (95% CI = 45.4%–52.1%) during weeks 17–28 ($p < 0.0001$). There were no statistically detectable group time interactions, suggesting that the rates of changing prevalence did not differ between rearing methods.

In contrast, the prevalence of resistance to chloramphenicol was relatively consistent across the first 16 weeks of the feeding period, but the samples collected during weeks 17–28 had lower prevalence of resistance. The LS mean resistance prevalence for chloramphenicol, controlling for rearing method, was 1.4% (95% CI = 0.8%–2.6%) during weeks 1–3 compared with 0.5% (95% CI = 0.2%–0.9%) during weeks 17–28 ($p = 0.01$). Similarly, for naladixic acid, the resistance prevalence was lowest at the end of the feeding period compared with the prevalence estimated from samples collected during the first 16 weeks of the feeding period. Controlling for rearing method, the LS mean prevalence of resistance to naladixic acid during weeks 1–3 was 1.2% (95% CI = 0.6%–2.5%) compared with 0.4% (95% CI = 0.2%–0.8%) during weeks 17–28 ($p = 0.04$). There were no statistically detectable group× time interactions, suggesting that rates of changing prevalence did not differ between rearing methods.

Recovery of S. enterica

S. enterica was recovered from 0.73% of fecal samples (93/12,760). There was no detectable difference between *Salmonella* isolation prevalences for natural and conventional cattle; 44 isolates were recovered from natural cattle and 49 were recovered from conventional cattle. Thirty-seven *Salmonella* isolates were serovar Agona, 18 were Muenchen, 10 were Newport, 6 were Cerro, 5 each were Anatum, and Oranienburg, 2 each were Bredeney, Give, and Montevideo, 1 each were Cubana, Dublin, Ohio, and 4,12:d, and 2 isolates were not recoverable after freezing. As might be expected, there appeared to be clustering of *Salmonella* shedding within pens of cattle even though shedding prevalence was very low among all pens. Over half of the isolates (53/93) were recovered from 5 pens of cattle, and the remaining 40 isolates were recovered from 21 pens of cattle. Most of the *Salmonella* isolates (93%, 87/93) were recovered in summer or fall months (June through November) compared with only 6.5% of *Salmonella* isolates that were recovered in winter or spring months (December through May). Isolates recovered from the pens with highest rates of recovery also tended to cluster by serotype and susceptibility phenotype. Approximately 53% of *Salmonella* isolates (49/93) were susceptible to all AMDs evaluated. Because of the very low recovery rate and also because of the strong clustering of recovery in pens of isolates with particular phenotypic and serotype patterns, it was not considered prudent to perform additional inferential analysis of *S. enterica* isolates.

Discussion

The findings of this study suggest that conventional production methods used in rearing feedlot cattle (including parenteral and in-feed use of AMDs) do not predictably or uniformly increase the prevalence of antimicrobial resistance among fecal NTSEC when compared with rearing methods used to produce branded "natural" beef products (i.e., beef products with a label claim that source cattle were not exposed to AMDs, hormone implants, or chemical anthelmintics). Overall, the prevalence of resistance among all NTSEC isolates was very low (<1% for 9 of 15 drugs, 1%–5% for 2 drugs, 6%–15% for 2 drugs, and >15% for only 1 drug). Although there were detectable differences in antimicrobial resistance in association with differences in rearing practices among NTSEC for four AMDs, the practical significance of these associations is not clear. For instance, although these differences in resistance prevalence existed, it was not clear what stimuli caused these changes to occur as quantitative measures of AMD exposures occurring during the feeding period were not associated with resistance prevalence. Most notably, parenteral exposure to enrofloxacin and florfenicol as measured at the pen level were not associated with differences in resistance prevalence in the most closely related drugs that were evaluated (naladixic acid, ciprofloxacin, or chloramphenicol).

It is possible that the impacts of different AMD exposures were overwhelmed by environmental exposures to the accumulated microbiome. We have previously found that both AMD treatment and short-term exposures to hospital environments were associated with detectable differences in resistance in fecal NTSEC (Dunowska *et al.*, 2006). Thus, the unmeasured exposure to resistant and nonresistant microbes in the local environment may have a bigger immediate impact than the production-related uses of AMDs. This may have been the reason that resistance prevalence for different AMDS showed detectable increases and decreases over time as the average resistance among GI flora equilibrated with the resistance among the environmental microbiome.

Another consideration regarding the practical significance of these differences is the clinical importance of the four drugs for which resistance differed. Although resistance among NTSEC isolates was most commonly detected against tetracycline and there was a 9% difference in the resistance prevalence between the two groups (34.5% vs. 43.5%), this class of drugs continues to be efficacious and useful in treatment of a variety of conditions in feedlot cattle (including respiratory disease) managed with conventional rearing practices (Schunicht *et al.*, 2002a, 2002b). Further, streptomycin, sulfamethoxazole, and chloramphenicol have not been the recent focus of major concern regarding antimicrobial resistance in humans. Resistance to other drugs was much less common, including the drugs that differed in resistance prevalence between rearing protocols (chloramphenicol, streptomycin, and sulfamethoxazole). Additionally, these differences between "natural" and "conventional" groups were small (0.7% vs. 1.4% for chloramphenicol, 11.3% vs. 14.2% for

streptomycin, and 10.0% vs. 13.1% for sulfamethoxazole, respectively) and it was not clear that these differences would affect treatment efficacy. Further, it was not clear whether differences of this magnitude in these drugs represent important trends in microbial ecology as it pertains to the development and persistence of antimicrobial resistance in bacterial populations.

Notably, there was a very low prevalence of resistance to drugs that have been of particular interest regarding zoonotic transmission of antimicrobial resistance to humans (e.g., potentiated penicillins, cephalosporins, quinolones, aminoglycocides) and there were no differences associated with the two rearing methods. Cephalothin resistance prevalence was intermediate ($\sim$4.8%), but resistance to later generation drugs was extremely rare ($\leq$0.2%). Interestingly, resistance prevalence among NTSEC isolates increased as the feeding period progressed despite the fact that none of these study cattle was exposed to tetracyclines or cephalosporins during the study period. In contrast, resistance to chloramphenicol and nalidixic acid decreased toward the end of the feeding period despite the therapeutic use of enrofloxacin and florfenicol in these cattle. Resistance prevalences for other drugs were low and did not change throughout the feeding periods.

Respiratory disease is the most common reason for which the feedlot cattle receive parenteral antimicrobial treatment. A national survey conducted by the USDA estimated that 13.5% of feedlot cattle were parenterally administered long-acting AMD (drugs with >24-hour effect) and that 15.4% received short-acting AMDs (drugs with <24-hour effect) at least once during the feeding period (USDA, 1995). A later study reported that about 10% of all feedlot cattle receive antimicrobials parenterally to prevent clinical manifestations of respiratory disease (USDA, 2000). Additionally, the USDA has estimated that over 80% of large feedlots (>1000 heads) use AMDs in feed or water as a health or production management tool and that nearly 55% of all feedlot cattle in the United States received AMDs in their feed at some time during the feeding period (USDA, 1995, 2000). The drugs most commonly used in this manner were tylosin and tetracycline compounds (chlortetracycline, oxytetracycline, tetracycline). Although this practice has been clearly shown to decrease the occurrence of clinical disease, producers receive an added benefit that cattle gain weight faster and more efficiently than untreated cattle (Nagaraja and Chengappa, 1998). Thus, oral AMDs are commonly fed to cattle in feedlots to promote production efficiency, but part of the means by which growth is enhanced is through the prevention of subclinical disease.

Although differences in antimicrobial susceptibility were not strongly associated with differences in rearing methods in this study, there was a large difference in feeding duration that was required for the cattle to obtain the same finish weight. Pens of cattle reared without hormone or AMD exposures required an average of 50 extra days in the feedlot compared with conventionally reared cattle. As noted previously, one of the three primary motivating factors for consumers to purchase "natural" or "organic" products is a perception that there are fewer ethical concerns (e.g., environmental or welfare concerns) related to production in comparison to conventional production methods (Hammitt, 1990; Grannis and Thilmany, 2000; Williams and Hammitt, 2000, 2001; Harper and Makatouni, 2002; Yiridoe *et al.*, 2005; Magkos *et al.*, 2006; Thilmany, 2006; Thilmany *et al.*, 2006). However, the much greater feeding time required to finish feeding the "natural" cattle enrolled in this study also represented a major difference in the amount of feed consumed as well as total fecal and urine output. Assuming that cattle reared without hormones or AMDs needed an average of 50 extra days to reach the same target weight of 545 kg and that cattle were fed $\sim$10.3 kg of dry matter per day, which is typical for feedlot cattle at this stage of production, this would be associated with an average of 511 kg more feed consumed per animal in comparison to cattle reared using conventional methods.

For the limited population of cattle enrolled in this study (4557 natural cattle), this represented a total of $\sim$2.3 million kg (5.1 million lbs) of additional feed. In addition, the extra fecal and urine output for these cattle is also considerable. Assuming that cattle of this size produce 30 kg (wet wt) of feces and 15 L of urine per day, each animal would produce 1500 kg of feces and 750 L of urine in 50 days. Therefore, for just the population of natural cattle enrolled in this study, an extra $\sim$6.8 million kg of feces and $\sim$3.4 million L of urine were produced in comparison to the cattle reared conventionally. The energy and water needed for production of this extra feed and the land space needed for disposal of this extra feces and urine are considerable. Thus, when considering the true benefit of raising cattle with restricted hormone and AMD exposure, the small differences in antimicrobial resistance that were seen in this study must be weighed against the very large differences in feed consumption and output of feces and urine.

This study focused on the effect of different management practices that occur in the feedlot setting, but it is important to consider that there were differences between cattle in the two study groups prior to their arrival at the feedlot. Production conditions related to future label claims on beef products (e.g., "Natural," "No Hormone Implants Used in Raising," "No Antibiotics Used in Raising") extended throughout the life of the animals, and thus, it is possible that differences between the two groups were affected by differences in AMD exposures that occurred prior to arrival at the feedlot. The observation that resistance prevalence for four drugs (chloramphenicol, tetracycline, sulfamethoxazole, and streptomycin) was greater among NTSEC recovered from conventionally reared calves in the first sampling period (1–3 weeks after arrival) than the resistance prevalence among isolates from natural calves is interesting. It is possible that this is a reflection of differences in resistance prevalence that predated arrival at the feedlot. However, because these samples were not taken immediately upon arrival, it is also possible that environmental and treatment exposures had a significant impact on these differences quite soon after arrival.

Additionally, although individual cattle can be certified to have no AMD treatments, this does not mean that this restricted exposure extends to other cattle that they have been in contact with and it does not make any certification about the environments where cattle were raised. In fact, after sick cattle from both groups were treated with AMDs they were all returned to their pens-of-origin. Thus, cattle from the same farms-of-origin or in the same pens could have received AMDs and this may have impacted the microbial ecology for other individual cattle even though other cattle have never been treated. There are no published data documenting the frequency that this management strategy (i.e., returning cattle to their home pen after treatment) is used in cattle destined to

produce beef with a restricted AMD exposure claim, but it is our impression that this is a common method for managing these cattle in feedlots that also use conventional production methods.

Although resistance prevalences may have decreased after arrival in cattle that were exposed prior to arrival to AMDs through treatment or their environment prior to arrival, it is not clear why exposures occurring prior to arrival would be associated with trends for increasing resistance prevalence after arrival. Studies evaluating the impact of treatment with florfenicol or ceftiofur on resistance in fecal NTSEC suggest that a single administration of these long-acting medications can have detectable, short-term impacts on the prevalence of resistance isolates (Berge *et al.*, 2005; Lowrance *et al.*, 2007). Although impact of treatment may not be detectable among individuals, the cumulative effect on populations (herds) and their environment may be substantive (Tragesser *et al.*, 2006).

It should be noted that cattle in both study groups had similar median numbers of parenteral AMD treatments per 100 heads of cattle in a pen. Subjectively, in comparison to treatment rates experienced with other feedlot cattle, treatment rates documented in this study were relatively low, indicating that the risk of infectious disease was low for most pens of cattle in comparison to some other feedlot cattle. Both of these factors may have affected the results of this study. Assuming that parenteral AMD exposures truly influence the prevalence of resistance among fecal NTSEC, the similarity in exposure might nullify differences between groups. However, subjective comparisons to other cattle that are being raised to produce "natural" and "conventional" beef products suggests that animals included in this study were comparable in terms of source characteristics, intrinsic host characteristics, disease history, environmental exposures, etc. As such, we believe that these results can be extrapolated to similar animals not included in this study. Cattle similar to those enrolled in this study are commonly fed using similar production methods throughout the United States and Canada, albeit cattle are much more commonly reared using conventional production methods. About half (49.8%) of all cattle fed in the United States are steers and heifers weighing <700 lbs on arrival at the feedlot, and 71% of all feedlots in the United States routinely feed this type of cattle (USDA, 1995).

It is not possible to say how measures of antimicrobial resistance in fecal NTSEC relate to human or animal health hazards. Fecal NTSEC have been commonly studied in investigations of antimicrobial resistance because they can be found in abundance in fecal material from all mammalian species and are relatively easy to recover from aerobic cultures. They also presumably provide a useful representation of the genetic pool of nonpathogenic bacterial flora that might contaminate foodstuffs. Although we did not investigate bacterial contamination of beef produced from these cattle, it might be presumed that NTSEC recovered from those products could have similar distributions of resistant bacteria. Although a recent study of enteric bacterial contamination of ground beef products produced from conventionally reared cattle and from cattle raised without antimicrobial exposure found that there were no differences in resistance prevalence between the two types of products for most AMDs that were evaluated (amoxicillin–clavulanate, ampicillin, ceftriaxone, gentamicin, kanamycin, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole), resistance prevalence was greater for ceftiofur and chloramphenicol among isolates recovered from conventional beef products (LeJeune and Christie, 2004). Interestingly, NTSEC isolates recovered from these ground beef products had a much higher prevalence of resistance when compared with fecal NTSEC isolates from this study, but other investigations of retail ground beef have reported resistance prevalences among NTSEC that were very similar to fecal isolates recovered in this study (NARMS, 2007).

Conclusions

The findings of this study suggest that conventional production methods used in rearing feedlot cattle (including parenteral and in-feed use of AMDs) do not predictably or uniformly increase the prevalence of antimicrobial resistance among fecal NTSEC when compared with rearing methods used to produce branded "natural" beef products (i.e., beef products with label claims that source cattle were not exposed to AMDs, hormone implants, or chemical anthelmintics). Although differences in resistance prevalence were detected between groups over time, in general these were not associated with recorded AMD exposures, which supports the hypothesis that these relationships are complex.

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Disclosure Statement

No competing financial interests exist.

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