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Influence of Therapeutic Ceftiofur Treatments of Feedlot Cattle on Fecal and Hide Prevalences of Commensal *Escherichia coli* Resistant to Expanded-Spectrum Cephalosporins, and Molecular Characterization of Resistant Isolates

John W. Schmidt,[a] Dee Griffin,[b] Larry A. Kuehn,[a] Dayna M. Brichta-Harhay[a]

U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska, USA[a]; Great Plains Veterinary Educational Center, School of Veterinary Medicine and Biomedical Sciences, University of Nebraska—Lincoln, Clay Center, Nebraska, USA[b]

In the United States, the bla_{CMY-2} gene contained within incompatibility type A/C (IncA/C) plasmids is frequently identified in extended-spectrum-cephalosporin-resistant (ESC^r) *Escherichia coli* strains from both human and cattle sources. Concerns have been raised that therapeutic use of ceftiofur in cattle may increase the prevalence of ESC^r *E. coli*. We report that herd ESC^r *E. coli* fecal and hide prevalences throughout the residency of cattle at a feedlot, including during the period of greatest ceftiofur use at the feedlot, were either not significantly different ($P \geq 0.05$) or significantly less ($P < 0.05$) than the respective prevalences at arrival. Longitudinal sampling of cattle treated with ceftiofur demonstrated that once the transient increase of ESC^r *E. coli* shedding that follows ceftiofur injection abated, ceftiofur-injected cattle were no more likely than untreated members of the same herd to shed ESC^r *E. coli*. Pulsed-field gel electrophoresis (PFGE) genotyping, antibiotic resistance phenotyping, screening for presence of the bla_{CMY-2} gene, and plasmid replicon typing were performed on 312 ESC^r *E. coli* isolates obtained during six sampling periods spanning the 10-month residence of cattle at the feedlot. The identification of only 26 unique PFGE genotypes, 12 of which were isolated during multiple sampling periods, suggests that clonal expansion of feedlot-adapted bla_{CMY-2} *E. coli* strains contributed more to the persistence of bla_{CMY-2} than horizontal transfer of IncA/C plasmids between *E. coli* strains at this feedlot. We conclude that therapeutic use of ceftiofur at this cattle feedlot did not significantly increase the herd prevalence of ESC^r *E. coli*.

Extended-spectrum cephalosporins (ESC) are critically important to human medicine and are frequently prescribed for the treatment of invasive *Escherichia coli* and *Salmonella enterica* infections (1–3). The bla_{CMY-2} gene, encoding the AmpC-like β-lactamase CMY-2, is frequently harbored by large (>120-kbp) incompatibility type A/C (IncA/C) plasmids in ESC-resistant (ESC^r) *E. coli* and ESC^r *S. enterica* strains isolated from human and animal sources in the United States (4–13). IncA/C plasmids are considered broad-host-range plasmids since they have been identified in many bacterial species, including *Aeromonas*, *Escherichia*, *Klebsiella*, *Photobacterium*, *Salmonella*, *Vibrio*, and *Yersinia* (14, 15). IncA/C plasmids possess conserved backbone sequences, but the sequences of genetic element insertions carrying antibiotic resistance genes are often divergent (14, 16–18). However, the insertions carrying genes conferring resistance to tetracyclines (*tetA*), phenicols (*floR*), and streptomycin (*aadA2*) are generally conserved between bla_{CMY-2} IncA/C plasmids harbored in *E. coli* and *S. enterica* hosts (17, 18). Thus, it has been hypothesized that commensal *E. coli* populations in the lower gastrointestinal systems of cattle may serve as a reservoir of bla_{CMY-2} IncA/C plasmids, which could then be transferred to more virulent food-borne pathogens, including *S. enterica* (12, 18).

Ceftiofur (TIO) is an ESC approved for use in cattle to treat several illnesses, including bovine respiratory disease complex. The critical importance of ESC to human medicine along with concerns that agricultural use of TIO may contribute to the occurrence of human ESC^r infections factored into the European Food Safety Authority recommendations to severely restrict or eliminate TIO use in animal agriculture (19). Injection with TIO has been demonstrated to transiently increase the fecal concentrations of ESC^r *E. coli* and bla_{CMY-2} in individual treated cattle (20–22). The long-term impact of therapeutic TIO injection of cattle on herd prevalence of ESC^r *E. coli* is unclear since only two studies have correlated TIO use (in dairy cattle herds sampled once or twice) to ESC susceptibilities of commensal *E. coli*. One study found an association between TIO use and isolation of *E. coli* with reduced susceptibility to the ESC ceftriaxone (23), while the other study found no association between extent of TIO use and ESC^r *E. coli* prevalence (24).

The factors contributing to long-term maintenance of ESC^r *E. coli* and the bla_{CMY-2} gene in the absence of TIO use are unclear. *In vitro* experiments have demonstrated that carriage of bla_{CMY-2} IncA/C plasmids imposes a fitness cost on the host bacteria, leading to the conclusion that long-term maintenance of IncA/C plasmids requires selective pressure (25). A mathematical model of ESC^r *E. coli* populations in cattle suggests that *E. coli* strains harboring bla_{CMY-2} IncA/C plasmids could persist during periods of low selective pressure even if they grow slower than other com-

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Address correspondence to John W. Schmidt, john.w.schmidt@ars.usda.gov.
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mensal *E. coli* strains if frequent horizontal transfer of bla_{CMY-2} IncA/C plasmids occurs or a sufficient fraction of *E. coli* bacteria ingested by cattle contain bla_{CMY-2} IncA/C plasmids (26). Both *E. coli* and *S. enterica* harboring bla_{CMY-2} IncA/C plasmids have been isolated from diverse animal hosts in disparate geographic locations with varied exposure to antibiotics; thus, an undefined combination of selective pressures, biological mechanisms, and population dynamics must ameliorate the fitness cost of bla_{CMY-2} IncA/C plasmid carriage (18). Isolation and characterization of ESC^r *E. coli* strains that persist in cattle production environments, in the absence of TIO use, is required to further our understanding of the factors that may contribute to the persistence of antibiotic resistance.

The population of 763 cattle used in this study was raised on pasture at the Roman L. Hruska U.S. Meat Animal Research Center (USMARC) from birth until weaning, when they were transferred to the on-site feedlot. The USMARC maintains detailed individual cattle health records, including all antibiotic treatments. This population of cattle was not treated with subtherapeutic levels of antibiotics. TIO was the preferred antibiotic for the treatment of bovine respiratory disease complex (shipping fever), infectious pododermatitis (foot rot), and infectious keratoconjunctivitis (pink eye). Typically, cattle at the USMARC feedlot are most susceptible to disease during the 4 to 6 weeks following weaning and introduction to the feedlot. The most concentrated use of therapeutic antibiotics occurs during this time, termed the "period of increased disease susceptibility." Following this period, occurrences of illness and antibiotic use typically decline (Shuna A. Jones [USMARC Veterinary Medical Officer], personal communication).

This population of cattle presented an opportunity for a longitudinal study on the effects of TIO use on the prevalence and persistence of ESC^r *E. coli*. This cattle population also presented an opportunity for the isolation and characterization of ESC^r *E. coli* prevalent in this cattle population during periods of limited TIO use. Thus, the goals of this study were to (i) determine the fecal and hide prevalences of ESC^r *E. coli* for the cattle population from feedlot arrival until shortly before harvest, (ii) determine the fecal and hide prevalences of ESC^r *E. coli* for the cattle injected with TIO during the period of increased disease susceptibility from the day of treatment to shortly before harvest, and (iii) characterize ESC^r *E. coli* cattle isolates obtained throughout the study with pulsed-field gel electrophoresis (PFGE) genotyping, antibiotic resistance phenotyping, plasmid size analysis, plasmid replicon typing, and screening for presence of the bla_{CMY-2} gene.

MATERIALS AND METHODS

Study population and cattle sampling. The study population consisted of 763 cattle (403 steers and 360 heifers) born between 22 March 2009 and 16 June 2009 at USMARC and raised on pasture at USMARC until weaned, when they were transferred to the USMARC feedlot between the dates of 25 September and 2 October 2009. The cattle then resided at the feedlot until July 2010, when they were transported to harvest. Detailed records of all antibiotics administered to study animals throughout their life span were maintained, and no antibiotics were included in feed. Samples were obtained during six periods, defined as follows: feedlot arrival (25 September 2009 to 2 October 2009), increased disease susceptibility (29 September 2009 to 30 October 2009), December 2009 (14 December 2009 to 22 December 2009), March 2010 (1 March 2010 to 5 March 2010), May 2010 (10 May 2010 to 13 May 2010), and July 2010 (13 July 2010 to 15 July 2010). Dates of the feedlot arrival and increased disease susceptibility periods overlapped since cattle were introduced to the feedlot (and samples taken) over 5 days (on 25, 29, and 30 September 2009 and 1 and 2 October 2009), while therapeutic TIO injections at the feedlot began on 29 September 2009, before the entire studied population arrived at the feedlot. Three classes of samples were obtained during this study and are described below, and the sampling scheme is illustrated in Fig. 1.

(i) Herd samples. At least 20% of the population of each pen was sampled during the feedlot arrival ($n = 153$), December 2009 ($n = 173$), March 2010 ($n = 178$), May 2010 ($n = 178$), and July 2010 ($n = 178$) periods. Cattle were selected for sampling using a random-number generator. During the period of increased disease susceptibility, 128 samples were obtained over 4 days (9, 16, 23, and 30 October 2009); on each of these days 32 cattle were sampled, two from each of the 16 pens containing the study population. Cattle previously injected with TIO were excluded from herd sampling during all six periods.

(ii) Cattle injected with TIO during the period of increased disease susceptibility. Fifty cattle were injected with TIO during the increased disease susceptibility period and were designated "IDS+TIO" cattle. Pre-TIO injection samples were obtained from 49 of these cattle. All cattle injected with TIO during the increased disease susceptibility period were held in a hospital pen until a follow-up health examination that occurred 3 to 8 days following injection. Post-TIO injection samples were obtained from all 50 IDS+TIO cattle during these follow-up health examinations. Following completion of the follow-up health examination, all cattle were returned to the pens they originally resided in, except for five cattle that remained in the hospital pen since they required additional observation. A total of 11 additional post-TIO injection samples were obtained from these five cattle during additional health examinations that occurred between 9 and 20 days after the first TIO injection. During the December 2009 period, samples were obtained from 49 of the 50 IDS+TIO cattle since one of the injected cattle died prior to the December 2009 sampling. During the March 2010, May 2010, and June 2010 periods, samples were obtained from 48 of the 50 IDS+TIO cattle since one of the injected cattle died prior to the March 2010 sampling.

(iii) Cattle injected with TIO at times other than during the period of increased disease susceptibility. Fifty-one cattle were injected with TIO at times other than during the period of increased disease susceptibility and were termed "Other+TIO" cattle. Nineteen cattle were injected with TIO prior to arrival at the feedlot, and samples were recovered from eight of these cattle during the feedlot arrival period. Fourteen additional cattle were injected with TIO between 1 November 2009 and 13 December 2009, for a total of 33 Other+TIO cattle, and all 33 were sampled during the December 2009 period. Six additional cattle were injected with TIO between 23 December 2009 and 28 February 2010, for a total of 39 Other+TIO cattle, and all 39 were sampled during the March 2010 period. Four additional cattle were injected with TIO between 6 March 2010 and 9 May 2010, for a total of 43 Other+TIO cattle, and all 43 were sampled during the May 2010 period. Eight additional cattle were injected with TIO between 14 May 2010 and 14 July 2010, for a total of 51 Other+TIO cattle, and all 51 were sampled during the July 2010 period.

Therapeutic antibiotic administration. During the life spans of the study cattle (March 2009 to July 2010), there were 157 occasions of therapeutic antibiotic administration (Table 1). One hundred thirty-seven cattle were injected with antibiotics; 16 cattle were injected with antibiotics on more than one occasion. During the life spans of the study cattle, there were 110 therapeutic TIO injections (Table 1). One hundred one cattle were injected with TIO; eight cattle received multiple TIO injections. The month with the highest number of TIO injections, 52, was October 2009. During November 2009, there were 14 TIO injections administered. From December 2009 through July 2010, 24 injections of TIO were administered. There were 47 administrations of other therapeutic antibiotics; the highest frequencies occurred in July 2009, with 16, and September 2009, with 13 (Table 1).

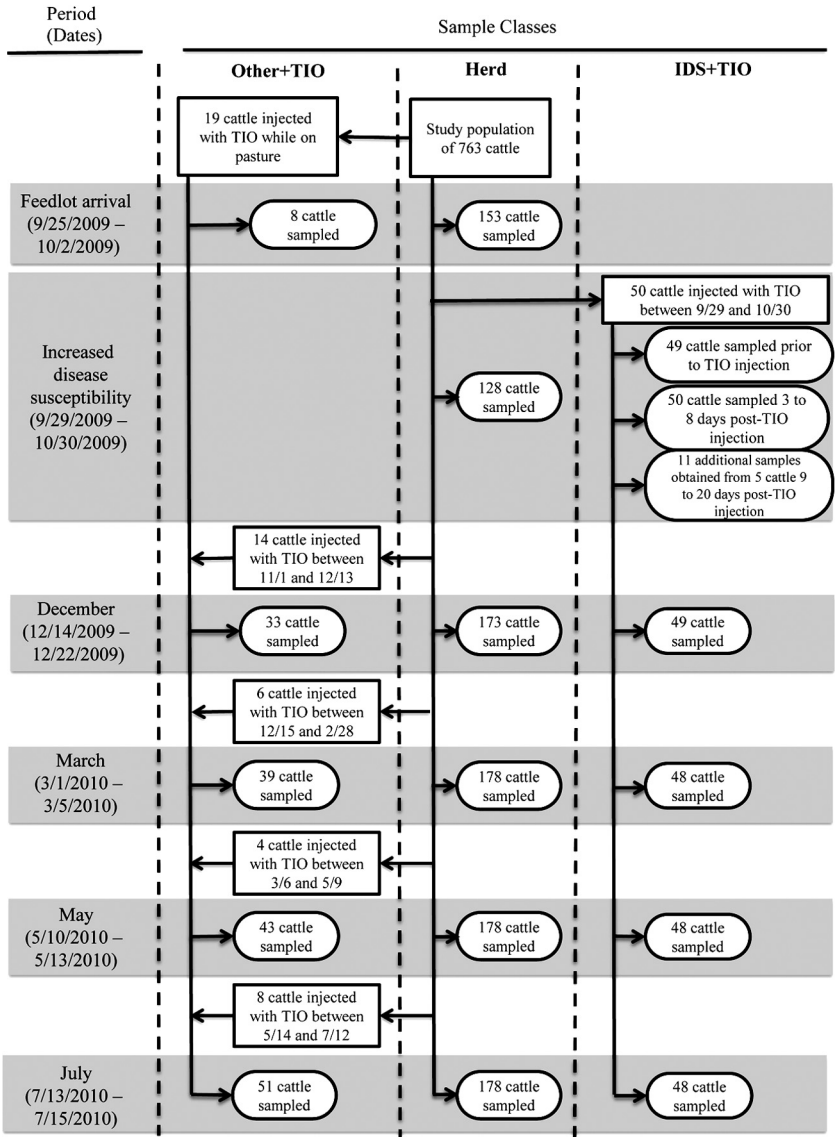

FIG 1 Flow diagram of sampling scheme. Gray boxes indicate sampling periods. Vertical dashed line lines delineate sample classes. Other+TIO, cattle injected with ceftiofur at times other than during the increased disease susceptibility period. IDS+TIO, cattle injected with ceftiofur during the increased disease susceptibility period.

Prevalence and enumeration of ESC^r *E. coli*. Sample collection occurred while cattle were restrained in a squeeze chute during vaccination, health examination, therapeutic treatment, or routine weighing. Fecal samples were collected by inserting a foam-tipped swab (catalog no. 10812-022; VWR International, Buffalo Grove, IL) 3 to 5 cm into the anus of each animal. Immediately following fecal sample collection, the swab was placed into 4 ml of tryptic soy broth (TSB) (Becton Dickinson, Sparks, MD). Hide samples (1,000 cm^2) were collected from each animal behind the right shoulder with a sterile sponge (Nasco, Fort Atkinson, WI) prewetted with 20 ml of buffered peptone water (BPW) (Becton Dickinson). Immediately following hide sampling, the sponge was placed into a sterile bag. Fecal and hide samples were collected by different people, and both changed gloves following each sample. Samples were processed within 4 h of sampling. Fecal samples were suspended by vortexing at maximum speed for 30 s. A 1-ml aliquot of suspended fecal matter was removed and serially diluted in TSB. Hide sponge samples were hand massaged for 15 s, and a 1-ml aliquot of suspension was removed and serially diluted in BPW. Selected dilutions were spiral plated onto MacConkey agar (Becton Dickinson, Sparks, MD) containing no antibiotics (MAC) and onto MacConkey agar supplemented with 4 mg $liter^{-1}$ of cefotaxime (MAC+CTX). Cefotaxime was obtained from Sigma Co. (St. Louis, MO). Plates were incubated overnight at 37°C. Pink to red colonies on MAC plates were enumerated as lactose-fermenting coliforms. Pink to red colonies on MAC+CTX were enumerated as presumptive ESC^r *E. coli*.

Confirmation of presumptive ESC^r *E. coli* isolates. Up to six presumptive ESC^r *E. coli* isolates per sample were streaked onto MAC+CTX plates and incubated at 37°C overnight. From each MAC+CTX streak plate, a single isolated pink to red colony was selected and streaked onto a Trypticase soy agar (TSA) plate (Becton Dickinson). One isolated colony from each TSA streak was inoculated into a 0.7-ml tryptic soy broth (TSB) (Becton Dickinson) culture contained in a 96-well block. Inoculated blocks were incubated overnight at 37°C, followed by the addition of glycerol to a final concentration of 15% to allow preservation at −80°C. Prior to freezing, each culture was stamped onto five 150-mm TSA plates with a 96-pin Boekel microplate replicator (Boekel Scientific, Feasterville, PA) to screen for antibiotic resistance. The five plates were supplemented

TABLE 1 Individual therapeutic antibiotic treatments administered to members of the studied herd of 763 cattle

Mo and yr	No. of injections of indicated antibiotic(s)[a]								Total no. of antibiotic injections	No. of animals injected with antibiotics
	Ceftiofur	Cloxacillin and oxytetracycline	Enrofloxacin	Florfenicol	Florfenicol and sulfadimethoxine	Oxytetracycline	Penicillin G	Tulathromycin		
March 2009	0	0	0	0	0	0	0	0	0	0
April 2009	0	1	0	1	0	0	0	0	2	2
May 2009	0	0	0	0	0	0	0	0	0	0
June 2009	1	0	0	0	1	0	0	0	2	2
July 2009	3	10	0	3	1	2	0	0	19	18[b]
August 2009	6	4	0	0	0	0	0	0	10	10
September 2009	10	8	0	0	0	3	2	0	23	23
October 2009	52	0	0	0	0	1	0	0	53	51[c]
November 2009	14	0	0	0	0	1	0	1	16	15[d]
December 2009	4	0	1	1	0	0	0	0	6	6
January 2010	0	0	0	0	0	5	0	0	5	5
February 2010	6	0	0	0	0	0	0	0	6	6
March 2010	3	0	0	0	0	0	0	0	3	3
April 2010	1	0	0	0	0	0	0	0	1	1
May 2010	3	0	0	0	0	0	0	1	4	4
June 2010	3	0	0	0	0	0	0	0	3	3
July 2010	4	0	0	0	0	0	0	0	4	4
Total over life spans of herd cattle (March 2009 to July 2010)	110	23	1	5	2	12	2	2	157	137[e]

[a] Two antibiotics listed in the same column indicate injection of the same animal with both antibiotics simultaneously.
[b] In July 2009 an animal was injected with ceftiofur and then 3 days latter injected with oxytetracycline.
[c] Two animals each received two ceftiofur injections in October 2009.
[d] One animal received two ceftiofur injections in November 2009.
[e] One hundred thirty-seven cattle were injected with antibiotics, 16 cattle were injected with antibiotics on more than one occasion.

with antibiotics as follows: no additional antibiotics, 32 mg liter^{-1} ampicillin (AMP), 4 mg liter^{-1} CTX, 64 mg liter^{-1} kanamycin (KAN), 32 mg liter^{-1} nalidixic acid (NAL), or 32 mg liter^{-1} tetracycline (TET). All antibiotics were obtained from Sigma Co. Plates were incubated overnight at 37°C, and isolates were grouped into categories based on their growth on the screened antibiotics. From each sample, at least one isolate from each category was selected for biochemical confirmation of *E. coli* and PFGE. Biochemical confirmation of *E. coli* was performed using the Sensititre broth microdilution system and Gram-negative identification plates (TREK Diagnostic Systems, Cleveland, Ohio) according to the manufacturers' instructions. Samples with one or more presumptive ESC^r *E. coli* isolates confirmed as *E. coli* were designated ESC^r *E. coli* prevalent.

PFGE. Pulsed-field gel electrophoresis (PFGE) analysis was performed according to the protocol developed by the Centers for Disease Control and Prevention (27). Agarose-embedded DNA was digested with XbaI (New England BioLabs, Beverly, MA). Banding patterns were either classified as unique or grouped into clusters based on ≥90% homology using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium), employing the Dice similarity coefficient with a 1.5% band position tolerance in conjunction with the unweighted-pair group method using arithmetic averages for clustering.

Antibiotic susceptibility determinations. Antibiotic susceptibility testing was performed using the Sensititre broth microdilution system and CMV1AGNF plates (TREK Diagnostic Systems) to determine the MIC for each of 15 antibiotic agents. The antimicrobials and breakpoints for resistance in this panel were as follows: amikacin (AMI), ≥64 μg ml^{-1}; amoxicillin-clavulanic acid (AMC) ≥32/16 μg ml^{-1}; AMP, ≥32 μg ml^{-1}; cefoxitin (FOX), ≥32 μg ml^{-1}; TIO, ≥8 μg ml^{-1}; ceftriaxone (AXO), ≥4 μg ml^{-1}; chloramphenicol (CHL), ≥32 μg ml^{-1}; ciprofloxacin (CIP), ≥4 μg ml^{-1}; gentamicin (GEN), ≥16 μg ml^{-1}; KAN, ≥64 μg ml^{-1}; NAL, ≥32 μg ml^{-1}; streptomycin (STR), ≥64 μg ml^{-1}; sulfisoxazole (FIS), ≥512 μg ml^{-1}; TET, ≥16 μg ml^{-1}; and trimethoprim-sulfamethoxazole (COT), ≥4/76 μg ml^{-1}. Isolates resistant to three or more classes of antibiotics were considered to be multidrug resistant (MDR). The antibiotic classes were as follows: aminoglycoside (AMI, GEN, KAN, and STR), β-lactam/β-lactamase inhibitor combination (AMC), cephem (FOX, TIO, and AXO), folate pathway inhibitor (FIS and COT), penicillin (AMP), phenicol (CHL), quinolone (CIP and NAL), and tetracycline (TET). The following organisms were used as quality control strains in the antimicrobial sensitivity assays: *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923.

Detection of the *bla*$_{CMY-2}$ gene and 18 plasmid incompatibility group replicons. Template DNA for PCR detection of the *bla*$_{CMY-2}$ gene and 18 plasmid incompatibility group (Inc) replicons was prepared by combining 10 μl of overnight culture of an isolated colony with 100 μl of BAX lysis buffer (DuPont Qualicon Inc., Wilmington, DE). Mixtures were incubated at 37°C for 20 min, followed by incubation at 95°C for 10 min. Lysates were then cooled to room temperature on ice. Lysates were subjected to PCR using the primers and conditions described by Kozak et al. (28) to determine the presence or absence of the *bla*$_{CMY-2}$ gene. Lysates were subjected to multiplex PCRs using the primers and conditions described by Johnson et al. (29) to determine the presence or absence of 18 plasmid Inc replicons (IncA/C, IncB/O, IncFIA, IncFIIA, IncFIB, IncFIC, IncFrep, IncHI1, IncHI2, IncI1, IncK/B, IncL/M, IncN, IncP, IncT, IncW, IncX, and IncY).

Plasmid size analysis. Plasmids were isolated using the method described by Kado and Liu (30). Isolated plasmids were subjected to agarose gel electrophoresis on 1% Tris-borate-EDTA agarose gels run for 4.5 h at 10 V cm^{-1}. Plasmid sizes were estimated relative to the BAC-Tracker supercoiled DNA ladder (catalog no. BT010950; Epicentre Technologies Corp., Madison, WI) and the supercoiled DNA ladder, 2 to 10 kb (catalog no. G6231; Promega Corp., Madison, WI).

Statistics. Period ESC^r *E. coli* prevalence values within a sample class (herd, IDS+TIO, or Other+TIO) and sample type (fecal or hide) were compared using Pearson's χ^2 with Bonferroni's correction for multiple comparisons, with P values of <0.05 considered significant. ESC^r *E. coli*

TABLE 2 Qualitative and quantitative evaluation of ESC^r *E. coli* for a cattle herd residing at a feedlot

Period	No. sampled	Fecal samples						Hide samples				
		% ESC^r *E. coli* prevalence (frequency)[a]	No. of samples in each class of log CFU/swab values					% ESC^r *E. coli* prevalence (frequency)[a]	No. of samples in each class of log CFU/100 cm^2 values			
			1.70 to 1.99	2.00 to 2.99	3.00 to 3.99	4.00 to 4.99	>4.99		1.30 to 1.99	2.00 to 2.99	3.00 to 3.99	>3.99
Feedlot arrival	153	3.9 (6) AB	2	2	1	0	1	15.0 (23) A	16	6	1	0
Increased disease susceptibility	128	5.5 (7) AB	2	4	0	1	0	11.7 (15) A	14	1	0	0
December 2009	173	2.9 (5) B	3	1	1	0	0	7.5 (13) AB	3	8	2	0
March 2010	178	1.7 (3) B	1	1	1	0	0	1.7 (3) B	3	0	0	0
May 2010	178	2.2 (4) B	3	1	0	0	0	17.4 (31) A	28	2	1	0
July 2010	178	11.2 (20) A	12	8	0	0	0	8.4 (15) A	15	0	0	0
Total	988	4.6 (45)	23	17	3	1	1	10.1 (100)	79	17	4	0

[a] Prevalence values in the same column that do not have a common letter are statistically significantly different ($P < 0.05$).

prevalence values from the same period were compared between sample classes (herd versus IDS+TIO and herd versus Other+TIO) using a two-tailed Fisher exact test, with P values of <0.05 considered significant. Comparisons of ESC^r *E. coli* prevalence values from the same period between sample types (fecal versus hide samples) were performed using a two-tailed Fisher exact test, with P values of <0.05 considered significant. All comparisons were performed using the Compare2 program of the WinPepi (ver. 11.7) package (31).

RESULTS

Herd prevalences and concentrations of ESC^r *E. coli*. Only cattle that had not received TIO injections were sampled to determine the herd prevalences and concentrations of ESC^r *E. coli*. When cattle arrived at the feedlot, the herd ESC^r *E. coli* prevalencesfecal prevalence was 3.9% (Table 2). Subsequent herd ESC^r *E. coli* fecal prevalences ranged from 1.7 to 11.2%, but none of these prevalences differed significantly from the prevalence at arrival. The highest herd fecal ESC^r *E. coli* prevalence (11.2%) occurred in July 2010 and was significantly higher ($P < 0.05$) than the December 2009, March 2010, and May 2010 (Table 2). Overall, 988 herd fecal samples were obtained during this study, and concentrations of ESC^r *E. coli* that were ≥2.00 log CFU/swab were obtained from 22 samples (2.2%) (Table 2). These ESC^r *E. coli* bacteria constituted only a small fraction of the total fecal lactose-fermenting coliforms shed; lactose-fermenting coliforms were enumerated from 984 of the 988 (99.6%) of the herd fecal samples, and mean lactose-fermenting coliform concentrations by sample period ranged from 4.84 to 6.00 log CFU/swab (data not shown).

When cattle arrived at the feedlot, the ESC^r *E. coli* hide prevalence was 15.0%, which was not different ($P \geq 0.05$) than subsequent hide prevalences except the 1.7% prevalence during March 2010, which was significantly lower ($P < 0.05$) than hide prevalences during all other periods except December 2009 (Table 2). Overall, ESC^r *E. coli* bacteria were enumerated on 100 hides, but the hide concentrations of ESC^r *E. coli* were <2.00 log CFU/100 cm^2 for 79 (79.0%) of these samples (Table 2). The concentrations of ESC^r *E. coli* on the hides were low in comparison to the concentrations of lactose-fermenting coliforms present on the cattle hides. Lactose-fermenting coliforms were enumerated from 980 of the 988 (99.2%) herd hide samples, with period mean concentrations of lactose-fermenting coliforms ranging from 3.11 to 4.66 log CFU/100 cm^2 (data not shown).

Prevalences and concentrations of ESC^r *E. coli* in feces and on hides of cattle injected with TIO during the period of increased disease susceptibility. During the period of increased disease susceptibility, 50 cattle were injected with TIO ("IDS+TIO" cattle). Pre-TIO injection samples were recovered from 49 of these IDS+TIO cattle, and the fecal prevalence of ESC^r *E. coli* was 8.2% (Table 3), which was not significantly different ($P = 0.50$) than the 5.5% herd fecal prevalence of ESC^r *E. coli* during the period of increased disease susceptibility (see Table S1 in the supplemental material). Post-TIO injection samples were obtained from all 50 IDS+TIO cattle at 3 to 8 days following TIO injection, and the fecal prevalence of ESC^r *E. coli* of 92.0% was significantly higher ($P < 0.05$) than the 8.2% prevalence pre-TIO injection (Table 3). The ESC^r *E. coli* fecal prevalence post-TIO injection for IDS+TIO cattle was also significantly higher ($P < 0.01$) than the 5.5% herd fecal prevalence of ESC^r *E. coli* during the period of increased disease susceptibility (see Table S1 in the supplemental material). For 11 additional samples obtained from five of these cattle during subsequent health examinations that occurred during the increased disease susceptibility period, the fecal prevalence of ESC^r *E. coli* was 90.9%. During the December 2009, March 2010, May 2010, and July 2010 sampling periods, the ESC^r *E. coli* fecal prevalence ranged from 0.0 to 6.1% (Table 3) and did not significantly differ ($P \geq 0.05$) from the corresponding herd ESC^r *E. coli* fecal prevalences during these periods (see Table S1 in the supplemental material). The fecal concentrations of ESC^r *E. coli* were ≥2.00 log CFU/swab for 52 of the 61 (85.2%) post-TIO injection samples obtained during the period of increased disease susceptibility, but fecal concentrations of ESC^r *E. coli* were ≥2.00 log CFU/swab for only 3 of the 193 (1.6%) samples obtained from these cattle during the subsequent December 2009, March 2010, May 2010, and July 2010 sampling periods (Table 3).

The 26.0% hide prevalence of ESC^r *E. coli* for IDS+TIO cattle sampled at 3 to 8 days post-TIO injection was higher than the 8.2% prevalence pre-TIO injection, but this difference was not statistically significant (Table 3). ESC^r *E. coli* hide prevalences for IDS+TIO cattle were not significantly different than the pre-TIO injection hide prevalence during the December 2009, March 2010, May 2010, and July 2010 periods but ranged from 24.5 and 20.4% during December 2009 and May 2010 to 0.0% during March 2010 and July 2010 (Table 3). Hide concentrations of ESC^r *E. coli* were

TABLE 3 Qualitative and quantitative evaluation of ESC^r *E. coli* for cattle injected with ceftiofur during the increased disease susceptibility period

Period	No. sampled	Fecal samples: % ESC^r *E. coli* prevalence (frequency)[a]	Fecal samples: No. of samples in each class of log CFU/swab values: 1.70 to 1.99	2.00 to 2.99	3.00 to 3.99	4.00 to 4.99	>4.99	Hide samples: % ESC^r *E. coli* prevalence (frequency)[a]	Hide samples: No. of samples in each class of log CFU/100 cm^2 values: 1.30 to 1.99	2.00 to 2.99	3.00 to 3.99	>3.99
Increased disease susceptibility, pre-ceftiofur injection	49	8.2 (4) B	0	4	0	0	0	8.2 (4) AB	2	2	0	0
Increased disease susceptibility, 3 to 8 days post-ceftiofur injection	50	92.0 (46) A	3	19	17	6	1	26.0 (13) A	10	1	2	0
Increased disease susceptibility, additional post-ceftiofur injection samples	11	90.9 (10)	1	3	5	1	0	27.3 (3)	1	2	0	0
December 2009	49	6.1 (3) B	1	1	0	1	0	24.5 (12) A	7	5	0	0
March 2010	48	0.0 (0) B	0	0	0	0	0	0.0 (0) B	0	0	0	0
May 2010	48	2.1 (1) B	0	1	0	0	0	20.8 (10) A	9	1	0	0
July 2010	48	2.1 (1) B	1	0	0	0	0	0.0 (0) B	0	0	0	0
Total	303	21.5 (65)	6	28	22	8	1	13.9 (42)	29	11	2	0

[a] Prevalence values in the same column that do not have a common letter are statistically significantly different ($P < 0.05$). Prevalences for increased disease susceptibility with additional post-ceftiofur injection samples were not included in statistical comparisons.

≥2.00 log CFU/100 cm^2 for only 13 of the 303 (4.3%) hide samples obtained from IDS+TIO cattle (Table 3). Pre-TIO injection, March 2010, May 2010, and July 2010 hide ESC^r *E. coli* prevalences for IDS+TIO were not significantly different ($P \geq 0.05$) from their corresponding herd ESC^r *E. coli* hide prevalences during these periods (see Table S1 in the supplemental material). However, the 26.0% hide prevalence of ESC^r *E. coli* for IDS+TIO cattle sampled at 3 to 8 days post-TIO injection was significantly higher ($P = 0.02$) than the herd ESC^r *E. coli* hide prevalence of 11.7% during the period of increased disease susceptibility (see Table S1 in the supplemental material). During December 2009, the 24.5% ESC^r *E. coli* hide prevalence for IDS+TIO cattle was significantly higher ($P < 0.01$) than the herd ESC^r *E. coli* hide prevalence of 7.5% (see Table S1 in the supplemental material).

Prevalences and concentrations of ESC^r *E. coli* in feces and on hides of cattle injected with TIO at times other than during the period of increased disease susceptibility. Cattle that had been injected with TIO at times other than during the period of increased disease susceptibility ("Other+TIO" cattle) were sampled during the feedlot arrival, December 2009, March 2010, May 2010, and July 2010 sampling periods. During the feedlot arrival period, samples were obtained from eight of the 19 cattle that had been injected with TIO while on pasture, and ESC^r *E. coli* was isolated from one fecal sample and one hide sample, which were not from the same animal (Table 4). The number of Other+TIO cattle sampled increased during each subsequent period, from 33 during December 2009 to 51 during July 2010, since TIO injections continued to occur during the residence of the herd at the feedlot (Table 1). ESC^r *E. coli* fecal prevalences for the samples obtained from December to July ranged from 0.0 to 9.8%. Fecal concentrations of ESC^r *E. coli* were ≥2.00 log CFU/swab for only two of the 174 (1.1%) samples obtained from Other+TIO cattle (Table 4). Prevalences of ESC^r *E. coli* on the hides of Other+TIO cattle were 9.1, 2.6, 25.6, and 3.9%, during December 2009, March 2010, May 2010, and July 2010, respectively (Table 4). Hide concentrations were ≥2.00 log CFU/100 cm^2 for only 3 of the 174 (1.7%) hide samples obtained from these cattle. ESC^r *E. coli* prevalences for Other+TIO cattle did not significantly differ ($P > 0.05$) from their corresponding herd prevalences during the December 2009, March 2010, May 2010, and July 2010 periods for both fecal and hide samples (see Table S2 in the supplemental material).

TABLE 4 Qualitative and quantitative evaluation of ESC^r *E. coli* for cattle injected with ceftiofur at times other than during the increased disease susceptibility period

Period	No. sampled	Fecal samples: % ESC^r *E. coli* prevalence (frequency)[a]	Fecal samples: No. of samples in each class of log CFU/swab values: 1.70 to 1.99	2.00 to 2.99	3.00 to 3.99	4.00 to 4.99	>4.99	Hide samples: % ESC^r *E. coli* prevalence (frequency)[a]	Hide samples: No. of samples in each class of log CFU/100 cm^2 values: 1.30 to 1.99	2.00 to 2.99	3.00 to 3.99	>3.99
Feedlot arrival	8	12.5 (1)	1	0	0	0	0	12.5 (1)	1	0	0	0
December 2009	33	3.0 (1) A	1	0	0	0	0	9.1 (3) AB	1	2	0	0
March 2010	39	0.0 (0) A	0	0	0	0	0	2.6 (1) B	1	0	0	0
May 2010	43	0.0 (0) A	0	0	0	0	0	25.6 (11) A	10	1	0	0
July 2010	51	9.8 (5) A	3	2	0	0	0	3.9 (2) B	2	0	0	0
Total	174	4.0 (7)	5	2	0	0	0	10.3 (18)	15	3	0	0

[a] Prevalence values in the same column that do not have a common letter are statistically significantly different ($P < 0.05$). Feedlot arrival prevalences were not included in statistical comparisons.

TABLE 5 ESC^r *E. coli* PFGE genotype prevalences

Sample type and period	No. of isolates	PFGE genotype frequency																								
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
Fecal samples																											
Feedlot arrival	7	1			6																						
Increased disease susceptibility	78	36		17		1	1	6	2		2	3		1		1	2	1		1	1	1	1	1			
December 2009	12					2			1		2	2		3	1				1								
March 2010	3	1				1									1												
May 2010	5	4	1																								
July 2010	28	24	1	1				1											1								
Total	133	66	2	18	6	4	1	7	3		4	5		4	2	1	2	1	2	1	1	1	1	1			
Hide samples																											
Feedlot arrival	25				22								2	1													
Increased disease susceptibility	42	13		14			7	1	1			1	1			1		1							1		1
December 2009	28					19			5		3															1	
March 2010	4												2		2												
May 2010	63	8	39	1			6			9																	
July 2010	17	14	1					1							1												
Total	179	35	40	15	22	19	13	2	6	9	3	1	5	1	3	1		1							1	1	1
Total (fecal and hide)	312	101	42	33	28	23	14	9	9	9	7	6	5	5	5	2	2	2	2	1	1	1	1	1	1	1	1

PFGE genotypes of ESC^r *E. coli* isolates. XbaI PFGE genotypes were obtained for 383 ESC^r *E. coli* isolates. Genotypes were obtained for 198 fecal isolates originating from 113 samples; thus, for 40 of the fecal samples more than one isolate was genotyped. Genotypes were obtained for 185 hide isolates originating from 158 samples; thus, more than one isolate was genotyped for 25 hide samples. Multiple XbaI PFGE banding patterns were identified from 39 of 65 samples that had more than one isolate genotyped (37 samples had 2 banding patterns, and 2 samples had 3 banding patterns). Seventy-one isolates had XbaI PFGE banding patterns identical to that of another isolate from the same sample and were considered redundant. Of the 312 nonredundant isolates, 133 originated from feces and 179 originated from hides. Overall, 26 unique XbaI PFGE banding patterns were identified, and these genotypes were assigned letters according to their prevalence (i.e., genotype A was most prevalent, followed by genotype B, etc.). Eight genotypes (S, T, U, V, W, X, Y, and Z) were identified from only one sample. Of the 18 genotypes identified from more than one sample, two (P and R) were identified exclusively from fecal samples, and two (I and L) were identified exclusively from hide samples (Table 5).

The overall most prevalent genotype, genotype A, was identified from 101 of the 271 (37.3%) ESC^r *E. coli*-positive samples. Genotype A was predominate in fecal samples, since it was identified in 66 of the 113 (58.4%) ESC^r *E. coli*-positive fecal samples. The next most prevalent fecal genotype (genotype C) was isolated from only 18 (15.9%) of the ESC^r *E. coli*-prevalent fecal samples. No single genotype predominated in ESC^r *E. coli*-prevalent hide samples, since genotypes B, A, D, and E were prevalent in 40 (25.3%), 35 (22.2%), 22 (13.9%), and 19 (12.0%) of the 158 ESC^r *E. coli*-positive hide samples, respectively. No genotype was isolated during all six sampling periods, but pattern A was prevalent during five of the six sampling periods, all except December 2009 (Table 5). Other genotypes prevalent during multiple sampling periods were C, E, L, and N, each being prevalent during three sampling periods. Genotypes B, F, G, H, J, K, and R were each prevalent during two sampling periods (Table 5).

Antibiotic resistance phenotypes of ESC^r *E. coli* isolates. Susceptibilities to 15 antibiotics were determined for the 312 nonredundant ESC^r *E. coli* isolates. All isolates were MDR, since all were resistant to at least three classes of antibiotics. All isolates were susceptible to AMI and CIP. Seven resistance phenotypes were identified. Resistance to at least AMC, AMP, FOX, TIO, AXO, CHL, STR, FIS, and TET (ACSSuTAuCfCtCx+ phenotype) was detected with 99.7% ($n = 311$) of isolates (Table 6). One isolate was susceptible to CHL but resistant to AMC, AMP, FOX, TIO, AXO, STR, FIS, and TET. For 13 of 14 genotypes with more than four isolates examined, the predominate resistance phenotype was observed for >75% of the isolates; genotype F was the only exception. Notably, all eight genotype F isolates with the ACSSuTAuCfCtCx + NAL resistance phenotype were from samples from the increased disease susceptibility period, while all six genotype F isolates with the ACSSuTAuCfCtCx + KAN + NAL resistance phenotype were from May 2010 samples (data not shown).

Prevalence of the bla_{CMY-2} gene, plasmid replicons, and plasmids of >80 kbp in ESC^r *E. coli* isolates. The bla_{CMY-2} gene was present in all 312 nonredundant ESC^r *E. coli* isolates. The plasmid replicons IncB/O, IncFIIA, IncFIC, IncHI1, IncHI2, IncK/B, IncL/M, IncP, IncT, IncW, and IncX were not detected from any isolate. The IncA/C replicon was the most prevalent replicon, being present in 69.2% ($n = 216$) of isolates (Table 7). Interestingly, all 97 isolates lacking the IncA/C plasmid replicon were genotype A (the IncA/C plasmid replicon was detected from only 5 of the 101 genotype A isolates). The next most prevalent plasmid replicon was IncY, present in 23.4% ($n = 73$) of the isolates. The IncFIB plasmid replicon was present in 20.2% ($n = 63$) of the isolates, and its presence was correlated with COT resistance, since 76.2% of the isolates with the IncFIB replicon were COT resistant and 82.8% of COT-resistant isolates possessed the IncFIB replicon (data not shown). One or more plasmids larger than 80 kbp were present in 68.9% ($n = 215$) of the 312 nonredundant ESC^r *E. coli* isolates (Table 7). Of the 97 isolates lacking a plasmid larger than 80 kbp, 95 were genotype A isolates. A single 4-kbp plasmid was

TABLE 6 Antibiotic resistance phenotypes of ESC^r *E. coli* isolates

PFGE genotype	No. of isolates	% of isolates with resistance to the indicated antibiotics[a]						
		AMC, AMP, FOX, AXO, TIO, STR, FIS, TET	AMC, AMP, FOX, AXO, TIO, CHL, STR, FIS, TET	AMC, AMP, FOX, AXO, TIO, CHL, NAL, STR, FIS, TET	AMC, AMP, FOX, AXO, TIO, CHL, STR, FIS, TET, COT	AMC, AMP, FOX, AXO, TIO, CHL, NAL, STR, FIS, TET, COT	AMC, AMP, FOX, AXO, TIO, CHL, KAN, NAL, STR, FIS, TET	AMC, AMP, FOX, AXO, TIO, CHL, GEN, KAN, STR, FIS, TET
A	101	0.0	97.0	2.0	1.0	0.0	0.0	0.0
B	42	0.0	0.0	97.6	0.0	0.0	2.4	0.0
C	33	0.0	3.0	0.0	90.9	6.1	0.0	0.0
D	28	0.0	100.0	0.0	0.0	0.0	0.0	0.0
E	23	0.0	13.0	0.0	0.0	87.0	0.0	0.0
F	14	0.0	0.0	57.1	0.0	0.0	42.9	0.0
G	9	0.0	100.0	0.0	0.0	0.0	0.0	0.0
H	9	0.0	100.0	0.0	0.0	0.0	0.0	0.0
I	9	0.0	77.8	22.2	0.0	0.0	0.0	0.0
J	7	0.0	85.7	0.0	0.0	14.3	0.0	0.0
K	6	0.0	100.0	0.0	0.0	0.0	0.0	0.0
L	5	0.0	0.0	0.0	0.0	0.0	0.0	100.0
M	5	0.0	100.0	0.0	0.0	0.0	0.0	0.0
N	5	0.0	100.0	0.0	0.0	0.0	0.0	0.0
O	2	0.0	0.0	0.0	100.0	0.0	0.0	0.0
P	2	0.0	100.0	0.0	0.0	0.0	0.0	0.0
Q	2	0.0	100.0	0.0	0.0	0.0	0.0	0.0
R	2	0.0	100.0	0.0	0.0	0.0	0.0	0.0
S	1	0.0	0.0	100.0	0.0	0.0	0.0	0.0
T	1	0.0	100.0	0.0	0.0	0.0	0.0	0.0
U	1	0.0	100.0	0.0	0.0	0.0	0.0	0.0
V	1	0.0	0.0	0.0	0.0	0.0	0.0	100.0
W	1	0.0	100.0	0.0	0.0	0.0	0.0	0.0
X	1	100.0	0.0	0.0	0.0	0.0	0.0	0.0
Y	1	0.0	100.0	0.0	0.0	0.0	0.0	0.0
Z	1	0.0	0.0	0.0	100.0	0.0	0.0	0.0
Total	312	0.3	59.9	17.3	10.9	7.4	2.2	1.9

[a] Antibiotics: AMC, amoxicillin-clavulanic acid; AMP, ampicillin; FOX, cefoxitin; AXO, ceftriaxone; TIO, ceftiofur; CHL, chloramphenicol; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; STR, streptomycin; FIS, sulfisoxazole; TET, tetracycline; COT, trimethoprim-sulfamethoxazole.

the only plasmid present in 89.1% ($n = 90$) of the genotype A isolates (data not shown).

DISCUSSION

A goal of this study was to determine the prevalence and levels of ESC^r *E. coli* in a feedlot cattle population when TIO was the preferred therapeutic antibiotic. TIO injection is known to transiently increase the fecal concentrations of ESC^r *E. coli* and bla_{CMY-2} in individual cattle before returning to levels observed prior to TIO injection (20–22). During the December 2009, March 2010, May 2010, and June 2010 sample periods, fecal prevalences of ESC^r *E. coli* for IDS+TIO or Other+TIO cattle were not significantly higher ($P \geq 0.05$) than the corresponding herd fecal prevalences (see Tables S1 and S2 in the supplemental material). This result demonstrates that once the transient increase of ESC^r *E. coli* shedding that follows TIO injection abates, TIO-injected cattle are no more likely than untreated members of the same herd to shed ESC^r *E. coli*. Two prior studies of the impact of TIO use on the prevalence of ESC^r *E. coli* in dairy cow herds had conflicting results, with one study finding an association and the other finding no association between the extent of TIO use and herd prevalence of *E. coli* with reduced susceptibility to ESC (23, 24). In this study, herd fecal and hide ESC^r *E. coli* prevalences when weaning calves arrived at the feedlot were not significantly lower ($P < 0.05$) than prevalences during any subsequent sampling period (Table 2). This suggests that the therapeutic use of TIO in feedlot cattle populations does not significantly increase the herd prevalence of ESC^r *E. coli*. However, we note that differences exist between the practices of the USMARC feedlot and those of commercial feedlots. Specifically, the USMARC cattle populations are "closed" (i.e., all feedlot cattle are born on center and raised until weaned on USMARC pastures, and replacement heifers are selected from USMARC populations), and introduction of cattle to USMARC feedlot occurs only twice per year when weaning calves are transferred to the feedlot. Thus, patterns of therapeutic antibiotic use in some commercial feedlots may differ from those in the USMARC feedlot.

E. coli strains associated with cattle are known to harbor antibiotic resistance genes, including genes encoding ESC^r (5, 32–36). These antibiotic resistance genes may be transmitted to human commensal and pathogenic bacteria, either by *E. coli* or when horizontally transferred to food-borne pathogenic bacteria, such as *S. enterica* (5, 37, 38). Therefore, fecal samples are typically examined to assess ESC^r *E. coli* or bla_{CMY-2} presence in cattle environments (20–24, 39). Hide samples were cultured for ESC^r *E. coli*, since hides have been demonstrated to harbor *E. coli* strains of fecal origin, including *E. coli* O157:H7 (40, 41). During this study, a total of 1,465 fecal and 1,465 hide samples were obtained, and bla_{CMY-2} positive ESC^r *E. coli* was prevalent on 10.9% ($n = 160$) of hides, which is significantly higher ($P < 0.01$) than the 8.0% ($n = 117$) prevalence in feces (data not shown). The 92.0% ($n = 46$)

TABLE 7 Prevalence of plasmids >80 kbp and replicons in ESC^r *E. coli* isolates

PFGE genotype	No. of isolates	% of isolates with at least one plasmid >80 kbp	% of isolates with indicated replicon						
			IncA/C	IncY	IncFIB	IncFrep	IncFIA	IncN	IncI1
A	101	5.9	5.0	12.9	4.0	0.0	2.0	4.0	4.0
B	42	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
C	33	100.0	100.0	39.4	84.8	48.5	3.0	6.1	3.0
D	28	100.0	100.0	100.0	0.0	3.6	100.0	0.0	0.0
E	23	100.0	100.0	4.3	78.3	0.0	0.0	0.0	0.0
F	14	100.0	100.0	0.0	0.0	85.7	0.0	0.0	0.0
G	9	100.0	100.0	100.0	0.0	0.0	0.0	11.1	0.0
H	9	88.9	100.0	11.1	44.4	22.2	0.0	0.0	0.0
I	9	100.0	100.0	0.0	0.0	66.7	0.0	100.0	0.0
J	7	100.0	100.0	0.0	28.6	14.3	0.0	0.0	0.0
K	6	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
L	5	100.0	100.0	60.0	20.0	100.0	40.0	20.0	0.0
M	5	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
N	5	100.0	100.0	0.0	100.0	0.0	100.0	0.0	0.0
O	2	100.0	100.0	50.0	0.0	0.0	50.0	0.0	0.0
P	2	100.0	100.0	0.0	0.0	50.0	0.0	0.0	0.0
Q	2	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0
R	2	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
S	1	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
T	1	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
U	1	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
V	1	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0
W	1	100.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0
X	1	0.0	100.0	0.0	0.0	100.0	0.0	0.0	0.0
Y	1	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
Z	1	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0
Total	312	68.9	69.2	23.4	20.2	14.4	12.5	5.8	1.6

fecal prevalence of ESC^r *E. coli* for IDS+TIO at 3 to 8 days post-TIO was significantly higher ($P < 0.01$) than the 26.0% ($n = 16$) hide prevalence for these cattle (Table 3). Genotype prevalences also differed between fecal and hide samples; for example, genotype B was the most prevalent hide genotype during May 2010 (isolated from 39 hides) but was isolated from only one May 2010 fecal sample (Table 5). Prevalence and genotype distribution differences between fecal and hide samples were expected, since fecal samples recover the *E. coli* population shed by the animal at the moment of sampling, while the hide sample obtained from the same animal likely contains a more broad representation of the overall *E. coli* population present in the feedlot environment at the time of sampling. These results demonstrate that hide sampling is a useful complement to fecal sampling for examination of the antibiotic resistance status of commensal *E. coli* in cattle feedlot environments.

To our knowledge, ESC^r *E. coli* isolated from a longitudinal study of a single cattle herd had not been subjected to genotypic analysis. In this study, we identified only 26 ESC^r *E. coli* genotypes, 12 of which were isolated during multiple sampling periods, and we identified a predominant genotype (A) which was isolated during five of the six sampling periods (Table 5). Our results suggest that clonal expansion of feedlot-adapted *E. coli* strains containing bla_{CMY-2} contributes to the persistence of the ESC^r *E. coli* in this cattle herd. We note that Daniels et al. identified 46 unique genotypes from 46 bla_{CMY-2} ESC^r *E. coli* isolates obtained from 14 cattle herds, suggesting that bla_{CMY-2} ESC^r *E. coli* populations between herds are diverse (7). Consideration of the results of this study, the study by Daniels et al., and the mathematical modeling of the persistence of bla_{CMY-2} ESC^r *E. coli* in cattle environments performed by Volkova et al. (26), it is likely that both conjugal transfer and clonal expansion contribute to bla_{CMY-2} IncA/C plasmid persistence in commensal *E. coli.* We note that *E. coli* has been demonstrated to survive for at least 6 months in cattle fecal pats (42, 43) and for at least 6 weeks on feedlot surfaces after the removal of cattle (44). Thus, we theorize that in individual feedlots, persistence of bla_{CMY-2} ESC^r *E. coli* occurs primarily by a cycle of ingested and shed clonal populations "adapted" to survive the specific stresses encountered in cattle and the environment at the individual feedlot level. We further theorize that conjugal transfer also occurs, ensuring that bla_{CMY-2} IncA/C plasmids are transferred to other receptive commensal *E. coli* strains, which are possibility more fit to survive when the environmental conditions change. Longitudinal studies of multiple cattle feedlots are required to test these theories of bla_{CMY-2} ESC^r *E. coli* persistence in cattle feedlots.

All genotype A isolates possessed the ACSSuTAuCfCtCx+ phenotype, typically conferred by large bla_{CMY-2} IncA/C plasmids, but 89.1% of these isolates had only one 4-kbp plasmid, which is not large enough to carry all of the resistance genes (bla_{CMY-2}, *tetA*, *floR*, *aadA2*, *sul1*, and *sul2*) typically harbored by bla_{CMY-2} IncA/C plasmids. Additionally, the IncA/C replicon was detected from only 5.0% of the genotype A isolates (Table 7). A few scenarios could explain these results. The integration of different genetic elements harboring the bla_{CMY-2} gene and the genes conferring the other antibiotic resistances could have occurred independently.

Indeed, presence of bla_{CMY-2} in the bacterial chromosome is not unprecedented, since a bla_{CMY-2} gene is contained within a chromosomally integrated SXT/R391-like element in a human clinical isolate of *Proteus mirabilis* (45). However, since all genotype A isolates had the ACSSuTAuCfCtCx+ phenotype, a more likely explanation is integration of a bla_{CMY-2} IncA/C plasmid into the *E. coli* chromosome. Failure to detect the IncA/C replicon can be explained if the plasmid site of integration was located between the annealing sites of the primers used to amplify the IncA/C replicon. Alternatively, the plasmid site of integration could be located elsewhere, but one of the primer annealing sites may have been altered by mutation, insertion, or deletion.

Concerns have been raised that therapeutic use of TIO in animal agriculture, including its use in feedlot cattle, contributes significantly to the increased occurrence of ESC^r infections in humans (2, 19, 46, 47). The isolation of bla_{CMY-2} ESC^r *E. coli* from U.S. retail beef supports these concerns, but the source of the ESC^r *E. coli* in these products is unknown (4). However, it is well established that the primary source of contamination of beef products with *S. enterica* and *E. coli* O157:H7 during processing are cattle hides with high levels of these pathogens (40, 41, 48, 49). The July 2010 hide samples were obtained within 2 weeks of harvest and approximate the population of ESC^r *E. coli* likely to be present on the hides of these cattle during processing. Herd prevalence of ESC^r *E. coli* on hides was 8.4% in July 2010, but ESC^r *E. coli* concentrations were $<$2.00 log CFU/100 cm^2 for all of these samples (Table 2). Thus, the ESC^r *E. coli* likely present on these cattle hides when sent to harvest was a very small fraction of the overall lactose-fermenting coliform population on the hides, since the herd prevalence of lactose-fermenting coliforms on hides during July 2010 was 100% and the mean concentration was 4.60 log CFU/100 cm^2 (data not shown). Complex factors, including contamination in lairage, can alter the microbial populations present on cattle hides at harvest (50, 51). Clearly, studies on the prevalence, concentrations, and genotypes of ESC^r *E. coli* on cattle hides when processing begins and in final products from the same processing location are required for a complete understanding of frequency and sources of the ESC^r *E. coli* contamination of beef products.

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