



ORIGINAL ARTICLE

Metaphylactic antimicrobial effects on occurrences of antimicrobial resistance in *Salmonella enterica*, *Escherichia coli* and *Enterococcus* spp. measured longitudinally from feedlot arrival to harvest in high-risk beef cattle

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Abstract

Aims: Our objective was to determine how injectable antimicrobials affected populations of *Salmonella enterica*, *Escherichia coli* and *Enterococcus* spp. in feedlot cattle.

Methods and Results: Two arrival date blocks of high-risk crossbred beef cattle ($n = 249$; mean BW = 244 kg) were randomly assigned one of four antimicrobial treatments administered on day 0: sterile saline control (CON), tulathromycin (TUL), ceftiofur (CEF) or florfenicol (FLR). Faecal samples were collected on days 0, 28, 56, 112, 182 and study end (day 252 for block 1 and day 242 for block 2). Hide swabs and *subiliac* lymph nodes were collected the day before and the day of harvest. Samples were cultured for antimicrobial-resistant *Salmonella*, *Escherichia coli* and *Enterococcus* spp. The effect of treatment varied by day across all targeted bacterial populations ($p \leq 0.01$) except total *E. coli*. Total *E. coli* counts were greatest on days 112, 182 and study end ($p \leq 0.01$). Tulathromycin resulted in greater counts and prevalence of *Salmonella* from faeces than CON at study end ($p \leq 0.01$). Tulathromycin and CEF yielded greater *Salmonella* hide prevalence and greater counts of 128ERY^R *E. coli* at study end than CON ($p \leq 0.01$). No faecal *Salmonella* resistant to tetracyclines or third-generation cephalosporins were detected. Ceftiofur was associated with greater counts of 8ERY^R *Enterococcus* spp. at study end ($p \leq 0.03$). By the day before harvest, antimicrobial use did not increase prevalence or counts for all other bacterial populations compared with CON ($p \geq 0.13$).

Conclusions: Antimicrobial resistance (AMR) in feedlot cattle is not caused solely by using a metaphylactic antimicrobial on arrival, but more likely a multitude of environmental and management factors.

KEYWORDS

antimicrobial resistance, *Enterococcus*, *Escherichia coli*, feedlot cattle, injectable antimicrobials, Metaphylaxis, *Salmonella*

Significance and Impact

At feedlot arrival, some cattle receive a metaphylactic antimicrobial. This practice potentially provides a reservoir for accumulation of antimicrobial-resistant *Escherichia coli*, *Salmonella*, and *Enterococcus* spp. in animals destined for human consumption. Our study is significant because it measures AMR longitudinally throughout the receiving and finishing phase of feedlot cattle.

INTRODUCTION

Antimicrobial resistance (AMR) is a global human-health problem in the 21st century (CDC, 2019a). The discovery of antimicrobials was a milestone for modern medicine; however, the development of novel antimicrobials has dramatically decreased because of fewer financial incentives for major pharmaceutical companies (Powers, 2004). Each year in the United States, there are approximately 2.8 million antimicrobial-resistant infections and nearly 35,000 related deaths (CDC, 2019a). Therefore, the preservation of antimicrobial efficacy is critical for the future of human health. The primary cause of AMR in human medicine is increased use associated with over-prescription and clinical misuse of antimicrobials; although, the potential exists for agricultural practices such as metaphylactic antimicrobial use in food animals to contribute to AMR in humans (Michael, Dominey-Howes, & Labbate, 2014).

Metaphylaxis is a common practice for U.S. cattle feeders and is defined as the use of antimicrobials to decrease the spread of infectious diseases within a population containing infected animals by treating the entire population (AVMA, 2021). High morbidity and mortality rates associated with bovine respiratory disease (BRD) represent a major financial loss for U.S. beef cattle feeders. Previous management factors put cattle at high risk for developing BRD. Those factors include cattle that are unvaccinated, not weaned, not castrated, comingled at auction markets, transported long distances and cattle exposed to other stressors before or at feedlot arrival (Callan & Garry, 2002; Hay et al., 2016). To help prevent and control the occurrence of BRD, approximately 21% of U.S. feedlot cattle receive a metaphylactic antimicrobial at feedlot arrival (USDA, 2011). Furthermore, common antimicrobials used for metaphylactic treatment of cattle belong to similar classes of antimicrobial drugs classified as critically important to human medicine (USDA, 2011; WHO, 2019).

As a result, cattle serve as a possible reservoir for bacteria to acquire resistance genes and be transferred to the human food chain.

Salmonella and *Escherichia coli* are specific bacteria of interest. Annually in the United States, the pathogen *Salmonella* causes approximately 1.35 million infections; 26,500 hospitalizations; and 420 deaths (CDC, 2022). Furthermore, pathogenic *E. coli* are responsible for approximately 205,000 infections, 2500 hospitalizations and 20 deaths per year in the United States (Scallan et al., 2011). Both *Salmonella* and *E. coli* can contaminate carcasses from hides during processing; however, *Salmonella* is also able to colonize the lymphatic system and contaminate ground beef via trimmings (Arthur et al., 2008; Bailey, Huynh, Govenlock, Jordan, & Jenson, 2017). Therefore, the objective of this study was to investigate the link between concentrations of antimicrobial-resistant *Salmonella*, *Enterococcus* and *E. coli* resulting from common metaphylactic antimicrobials.

MATERIALS AND METHODS

This experiment was conducted at the Texas Tech University research feedlot from October 2020 to August 2021 and was approved by the Texas Tech University Animal Care and Use Committee (approval number 20039–04). The temperature during the study ranged from -17.7°C to 42.2°C with a total precipitation of 499.5 mm and average relative humidity of 44%.

Animals

Two hundred forty-nine high-risk cattle containing 13 bulls and 236 steers with an average initial body weight (BW) of 244 kg (± 25 kg SD) were sourced from multiple locations and blocked by arrival date. Block 1 arrived on October 22, 2020 and contained 1 bull and 123 steers ($n = 124$) purchased from an auction market in Dalhart, TX travelling approximately 322 km. Block 2 arrived on December 2, 2020 and contained 12 bulls and 113 steers ($n = 125$) purchased from an auction market in West Plains, MO travelling approximately 1186 km.

Treatments

A generalized complete block design was used in which pens were assigned to one of four metaphylactic

antimicrobial treatment groups. Metaphylactic treatments were administered once on day 0 and consisted of a 5 ml sterile saline negative control (**CON**), florfenicol (**FLR**; Nuflor; Merck Animal Health, Kenilworth, NJ; 6 ml 45 kg bw^{-1}), ceftiofur (**CEF**; Excede; Zoetis, Parsippany, NJ; 1.5 ml 45 kg bw^{-1}) and tulathromycin (**TUL**; Draxxin; Zoetis; 1.1 ml 45 kg bw^{-1}). These metaphylactic antimicrobials were chosen because they represent the most frequently used antimicrobials in feedlot beef production, and are classed similarly to antimicrobials categorized as critically important to human medicine by the WHO (USDA, 2011; WHO, 2019). Each antimicrobial was administered according to label instruction and was assigned a postmetaphylactic interval (PMI) according to veterinary consultation. The PMI was 3 day for florfenicol, 5 day for ceftiofur and 7 day for tulathromycin; however, control cattle injected with sterile saline had no PMI and were eligible for therapeutic antimicrobial treatment on day 0.

Animal management

Detailed methods on animal husbandry, BRD treatment and management are reported in Coppin (2021). Briefly, cattle were received in soil-surface pens where hay and water were offered. Then, cattle were vaccinated and administered an anthelmintic approximately 24 h after arrival. Following processing in each block, cattle were sorted by BW into four groups containing 31 or 32 animals. Then, experimental treatments were randomly assigned to each group and administered approximately 48 h after arrival on study day 0. Cattle were housed in sections of three pens containing 10 or 11 animals per pen, and each treatment group was separated by an empty pen during the receiving period. The receiving diet contained approximately 65% concentrate and was fed at approximately 1% of BW. Cattle were monitored daily for visual signs of BRD and were eligible for up to three additional therapeutic antimicrobial treatments as needed. Therapeutic treatment antimicrobials consisted of enrofloxacin (Baytril 100; Bayer Animal Health), tildiprosin (Zuprevo; Merck Animal Health) and danofloxacin (Advocin; Zoetis) with a 3 day, 7 day and 0 day post-treatment interval (PTI) respectively.

On day 38 for block 1 and day 45 for block 2, cattle from the same experimental treatment were sorted into slatted concrete-surfaced pens containing four animals per pen. Metaphylactic treatment groups were maintained and separated by an empty pen. Cattle were revaccinated on day 28 and implanted twice throughout the study. Cattle were transitioned from the receiving diet using a 4-step gradual process with a 7-day adaptation period for each step as concentrate was increased from 65, 75, 85 and 90% of the diet. The finishing diet was based on steam-flaked

corn and contained 90% concentrate as well as 30 g ton^{-1} monensin sodium (285 mg hd^{-1} day^{-1} ; Rumensin 90; Elanco Animal Health). The National Academies of Sciences and Medicine Nutrient Requirements for Beef Cattle Guide (2016) was used to ensure diets were formulated to meet or exceed nutrient requirements of growing and finishing beef cattle.

Faecal sampling procedures

Faecal grab samples were collected on days 0, 28, 56, 112, 182 and study end (day 252 for block 1 and day 242 for block 2). Cattle were restrained in a chute (Silencer; Moly Manufacturing) and a new shoulder length obstetrics glove was donned before each animal was sampled. Faecal samples were collected by cupping the hand and removing any faecal material present in the terminal 15 cm of the rectum. Next, the sample was placed into a clean closable plastic bag. If no faeces were collected, the glove was then inverted and placed into the plastic bag. Each bag was sealed and placed into a cooler with ice, and coolers were shipped to the USDA-ARS, United States Meat Animal Research Center (USMARC) in Clay Center, NE for microbial analysis. Upon arrival, faecal samples were stored overnight at 4°C before being processed.

Faecal sample processing for *Escherichia coli* and *Enterococcus* spp.

Microbial analysis of faecal samples for *E. coli* and *Enterococcus* was conducted following previously described procedures by Agga, Schmidt, and Arthur (2016). Briefly, 10 g of each sample was placed into a filter bag and mixed with 90 ml of Tryptic Soy Broth (TSB; Becton, Dickinson and Company) containing 100 mM potassium phosphate buffer (18 mM KH_2PO_4 and 82 mM K_2HPO_4 , pH 7.2; Sigma) and mixed well by hand massage. Next, 500 μl of the solution was subsampled for AMR bacterial enumerations. The remaining solution was enriched for 8 h at 37°C to determine AMR bacteria and pathogen prevalence. Bacterial enumerations were conducted by spiral plating (WASP Touch; Don Whitley Scientific) serial dilutions of 50 μl from each subsample onto CHROMagar *E. coli* (DRG International Inc.), CHROMagar ECC (DRG International Inc.) or CHROMagar Orientation (DRG International Inc.). Targeted AMR populations were total, tetracycline-resistant (TET^{R} , 32 mg L^{-1} tetracycline), trimethoprim-sulphamethoxazole-resistant (COT^{R} , 4 mg L^{-1} trimethoprim and 76 mg L^{-1} sulphamethoxazole) and cefotaxime-resistant (CTX^{R} , 2 mg L^{-1} cefotaxime) *E. coli*; total, erythromycin-resistant (ERY^{R} , 8 mg L^{-1}

erythromycin) and highly ERY^R (128 mg L⁻¹ erythromycin) *Enterococcus*. Resistance levels were determined from the National Antimicrobial Resistance Monitoring System and the suggested concentration of each antimicrobial was added to the selective agar plates (CDC, 2019b). However, an exception was used to select for *Enterococcus* populations highly ERY^R at 128 mg L⁻¹. *Enterococcus* and *E. coli* prevalence from samples not confirmed enumerable was determined by direct plating 20 µl of each enrichment onto individualized selective media agar plates. All plates were incubated for 8 h at 37°C. Morphologically distinct colonies on each agar plate were counted for enumeration or considered presumptive positive for prevalence. All presumptive colonies were picked and confirmed by PCR using *uidA* gene for *E. coli* and *soda* for *Enterococcus* (Jackson, Fedorka-Cray, & Barrett, 2004; Molina et al., 2015).

Faecal sample processing for *Salmonella*

Microbial analysis of faecal samples for *Salmonella* was conducted similarly to that of Agga et al. (2016). Enumeration was conducted using a direct plating technique (detection limit ≥ 200 colony-forming units [CFU] g⁻¹ of faecal sample), and a WASP Touch (Don Whitley Scientific) was used to spiral plate 50 µl of each pre-enriched, TSB-diluted faecal sample onto Xylose Lysine Deoxycholate agar (XLD; Becton, Dickinson and Company Difco™). Enriched faecal samples from the procedure described earlier were used to determine faecal prevalence of *Salmonella*. Briefly, 500 µl of phosphate-buffered saline with Tween (PBS Tween; Sigma Chemicals) and 10 µl anti-*Salmonella* magnetic beads (Applied Biosystems) were placed into deep-well 96-well blocks. Then, 500 µl of each enriched sample was transferred to individual wells, and a vibrating VWR Incubating Micro Plate Shaker (VWR International) was used to mix beads from each enrichment sample for 15 min at room temperature. After mixing, immunomagnetic beads were removed and washed two times in PBS-Tween using a Kingfisher 96 robotic processor (Thermo Life Sciences/Fisher Scientific) and beads were eluted into 100 µl of PBS-Tween. Lastly, a 50 µl aliquot of the bead-bacterial complex was transferred to 5 ml of RVS broth (Becton, Dickinson and Company) and enriched overnight at 42°C. A 10 µl loop of the RVS secondary enrichment was plated onto XLD, XLD-tet (32 mg L⁻¹ tetracycline) or XLD-ctx (2 mg L⁻¹ cefotaxime) agar, and the plates were incubated at 37°C overnight.

Hide swab collection and processing

On the day before harvest (day 252 for block 1 and day 242 for block 2), hide swabs were collected using a sponge

(Nasco Whirl-Pak) pre-wet with 10 ml of TSB. The sponge was removed from the TSB solution and a 1000 cm² section directly behind the shoulder of each animal was scrubbed before the sponge was put back into the bag (Nasco Whirl-Pak) and sealed. Hide swabs were then transported back to the USDA-ARS laboratory near Lubbock, TX for processing. After an additional 90 ml of TSB was added to each sample bag, hide swab samples were incubated at 37°C for 6 h. Then, sponges were massaged, and the suspension was streaked with a 10-µL loop onto XLD agar and BGA agar containing novobiocin (25 µg mL⁻¹). Separately, 1 ml of hide swab suspension was put into a 1:10 dilution of Rappaport Vassiliadis enrichment broth, then vortexed and incubated at 42°C overnight. The enrichment was then streaked with a 10-µL loop onto XLD agar and BGA agar containing 25 µg mL⁻¹ of Novobiocin. All XLD and BGA agar plates were incubated at 37°C for 24 h. Phenotypic colony re-streaks were confirmed via latex agglutination (*Salmonella* Latex Kit; Oxoid). Two phenotypic isolates were selected from positive enrichment plates and placed into a 1:10 dilution of glycerol to TSB. Isolates were frozen at -80°C for serotyping.

Subiliac lymph node collection and processing

Block 1 cattle were shipped to a commercial abattoir in June 2021 to be harvested. Likewise, block 2 cattle were shipped to the same commercial abattoir in August 2021. Trained personnel from Texas Tech University collected and tracked harvest order using animal tag numbers. A convenient sample of 58 *subiliac* lymph nodes was collected from block 1, and 105 were collected from block 2 (163 total) to be analysed for lymph node prevalence of *Salmonella*. As they were collected, each lymph node was placed into an individual plastic bag containing a tag matching the harvest order before being placed into a cooler with ice. Then, the lymph nodes were transported to the USDA-ARS Livestock Issues Research Unit near Lubbock, TX where they were sorted by ascending number and stored at 4°C overnight.

The next day, lymph nodes were aseptically de-nuded, weighed and sterilized in a boiling water bath for 3 s. Then, approximately 25 g of each lymph node was placed into a lateral filtered stomacher bag (Seward Laboratory Systems Inc.) and pulverized with a rubber mallet (Arthur et al., 2008). Phosphate-buffered saline (PBS; Thermo Fisher Scientific) was added to achieve a 1:10 dilution. The mixture was then homogenized using a stomacher machine (Stomacher® 400 Circulator; Seward Laboratory Systems Inc.) for 2 min at 2300 rpm. From the homogenate, a 100 µl aliquot was collected

and spiral plated using and Eddy Jet 2 W (Neutec Group Inc) onto Xylose Lysine Deoxycholate (XLD; Becton, Dickinson and Company Difco™) and Brilliant Green Agar (BGA; Becton, Dickinson and Company Difco™) containing novobiocin ($25 \mu\text{g mL}^{-1}$). Next, plates were incubated at 37°C for 24 h. Following incubation, plates were counted using an automated colony counter (Sphere Flash®; IUL Instruments). From the lymph node homogenate, an additional 1 ml was placed into a 1:10 dilution of Rappaport Vassiliadis enrichment broth, then vortexed and incubated at 42°C overnight. Likewise, another 1 ml of the lymph node homogenate was placed into a 1:10 dilution of Tetrathionate Broth with iodine, then vortexed and incubated at 37°C overnight. After incubation, enrichments were streaked with a $10\text{-}\mu\text{L}$ loop onto XLD agar and BGA agar containing novobiocin ($25 \mu\text{g mL}^{-1}$). Then, the plates were incubated at 37°C for 24 h. Additionally, XLD plates were left at room temperature for another 24-h post-incubation to allow additional growth and phenotype development. Phenotypic colonies were streaked onto fresh agar and confirmed by latex agglutination (Oxoid *Salmonella* Latex Kit). Finally, two phenotypic isolates were selected from positive enrichment plates and put into a 1:10 dilution of glycerol to Tryptic Soy Broth (TSB; Becton, Dickinson and Company Bacto™). Isolates were then frozen at -80°C for later serotyping.

Statistical analysis

Data expressed in colony-forming units (CFU/g) were converted using a log transformation (\log_{10} CFU g^{-1} of faeces) for bacterial concentration analyses. A lower limit for detection of enumeration was set at 200 CFU g^{-1} or $2.3 \log_{10}$ CFU g^{-1} . For nonenumerable, enriched samples, a value of 0.5 CFU g^{-1} was used for prevalence negative samples and a value of 100 CFU g^{-1} was used for prevalence positive samples. The PROC MIXED procedure of SAS (SAS Inst. Inc.; version 9.4) was used for analysis of faecal data. Individual animal served as the experimental unit with fixed effects of metaphylactic treatment, time and metaphylactic treatment \times time interaction and the random effect of arrival block. The Kenward Roger adjustment was used to correct the degrees of freedom for unequal experimental units among treatments. Animal nested within pen was the subject of the repeated measures and was included to control for any variation that occurred throughout the study. Several covariance structures were tested, but the autoregressive 1 resulted in the smallest Akaike and Schwarz Bayesian criteria and was considered the most appropriate for analysis. Because of numerous metaphylactic treatment \times time interactions simple effect least

square means are presented graphically, and a p -value of 0.05 was used to determine significance.

The PROC GLIMMIX procedure of SAS was also used to analyse hide swab and lymph node *Salmonella* prevalence data as binomial proportions. Once again, individual animal served as the experimental unit. Metaphylactic treatment was the fixed effect and block served as a random effect. The Kenward Roger adjustment was used to correct the degrees of freedom for an unequal number of observations per treatment. Simple effect least squares means are presented in tabular form, and a p -value of 0.05 was used to determine significance.

RESULTS

Therapeutic treatments following metaphylaxis

As reported by Coppin (2021), 58.8% of control, 26.3% of tulathromycin, 26.3% of ceftiofur and 45.2% of florfenicol treatment groups in the current study received therapeutic enrofloxacin for treatment of BRD. Additionally, 29.3% of control, 3.3% of tulathromycin, 8.44% of ceftiofur and 14.5% of florfenicol treatment groups were administered therapeutic tildipirosin for a second treatment of BRD. Lastly, 7.4% of control, 0.0% of tulathromycin, 0.0% of ceftiofur and 1.6% of florfenicol treatment groups received therapeutic danofloxacin for a third treatment of BRD.

Total *Salmonella* counts and prevalence from faecal samples

A treatment \times day interaction was detected for total *Salmonella* concentrations ($p < 0.01$; Figure 1a). However, on day 0, there were no differences for the concentrations among metaphylactic treatment groups ($p \geq 0.36$). On day 28, the TUL treatment had greater concentrations than CON, CEF and FLR ($p < 0.01$). Furthermore, concentrations among CON, CEF and FLR treatments were not different on day 28 ($p \geq 0.21$). On day 56, concentrations of both TUL and CON treatments were greater than CEF and FLR ($p < 0.01$). Additionally, on day 56, concentrations for the FLR treatment were greater than CEF treatment ($p < 0.05$). On day 112, the CON treatment had greater concentrations than CEF and FLR ($p \leq 0.03$) but was not different from TUL ($p = 0.16$). On day 182, concentrations for the CON treatment were greater than TUL, CEF and FLR ($p \leq 0.01$). *Salmonella* faecal concentrations for TUL, CEF and FLR treatments were not different on day 182 ($p \geq 0.17$). Lastly, on the day before harvest, concentrations for the TUL treatment were greater than

CON and FLR treatments ($p \leq 0.01$) but not different from CEF ($p = 0.25$). Enriched faecal samples were also tested for *Salmonella* resistant to either tetracyclines or

third-generation cephalosporins; however, no *Salmonella* resistant to either antibiotic was detected.

Furthermore, there were differences in faecal *Salmonella* concentrations within the treatments over time. Unlike other treatments, TUL *Salmonella* concentrations increased from day 0 to 28 ($p < 0.01$), decreased from day 56 to day 112 ($p = 0.04$) and increased again from day 182 to study end ($p \leq 0.03$). In contrast to other treatments, there was a decrease in *Salmonella* concentration in CON from day 182 to study end ($p < 0.01$).

A treatment \times day interaction was detected for prevalence of *Salmonella* ($p < 0.01$; Figure 1b). There were no differences in prevalence among treatments on day 0 and 112 ($p \geq 0.16$). On day 28, TUL treatment had 34.4, 38.2 and 36.8% greater prevalence compared to CON, CEF and FLR treatments ($p \leq 0.01$) respectively. On day 56, prevalence for the CON treatment was 33.1% greater than CEF treatment ($p < 0.01$) and 19.1% greater than FLR treatment ($p = 0.03$). Furthermore, *Salmonella* prevalence for the TUL treatment was 45.2% greater than CEF treatment ($p < 0.01$) and 31.1% greater than FLR treatment ($p < 0.01$). On day 182, the CON treatment had 18.3, 24.7 and 31.5% greater prevalence compared to TUL, CEF and FLR treatment ($p \leq 0.04$) respectively. Lastly, at study end, *Salmonella* prevalence for the TUL treatment was 26.5% greater ($p < 0.01$) than the CON treatment and 23.8% greater than FLR treatment ($p = 0.01$).

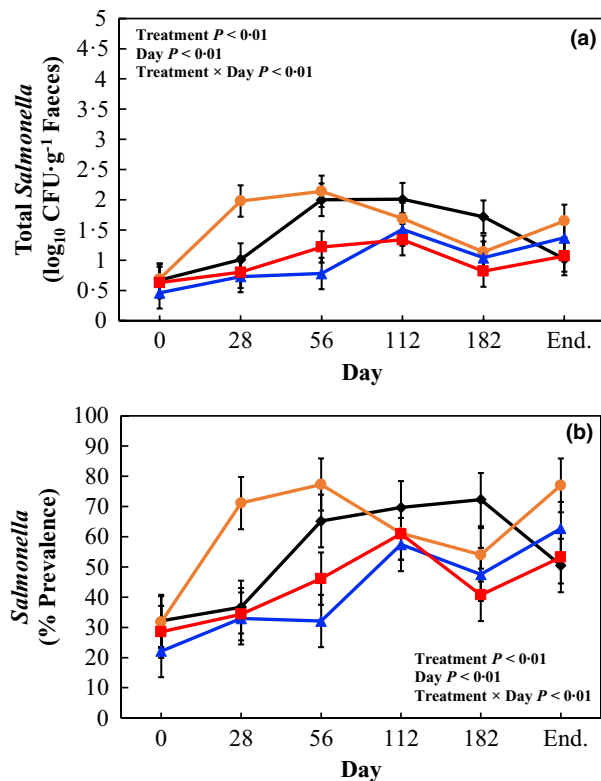


FIGURE 1 (a) \log_{10} CFU counts g^{-1} faeces of *Salmonella* plated on agar without antimicrobial supplementation and (b) Percent prevalence of *Salmonella* plated on agar without antimicrobial supplementation in faecal samples collected from cattle given metaphylactic antimicrobial treatments of tulathromycin (Draxxin; orange line; \bullet), ceftiofur (Excede; blue line; \blacktriangle) or florfenicol (Nuflor; red line; \blacksquare) compared to cattle not given a metaphylactic antimicrobial 9 (Control; black line; \blacklozenge) on day 0. Error bars represent standard error of the mean. Study end was on day 252 for block 1 and day 242 for block 2.

Prevalence of *Salmonella* in hide swabs and lymph nodes

Differences were detected for *Salmonella* prevalence in hide swabs collected from cattle on the final sample date ($p < 0.01$; Table 1). The total proportion of *Salmonella* on the hides for TUL-treated cattle were 53.0, 19.0 and 37.6% greater compared to the CON, CEF and FLR treatments ($p \leq 0.03$) respectively. Additionally, the proportion of

TABLE 1 *Salmonella* prevalence of finishing cattle when hide swabs were collected the day before harvest and lymph nodes were collected at harvest

Item	Treatments*				SEM	p-value
	CON	TUL	CEF	FLR		
Animals, <i>n</i>	56	55	57	60	–	–
Hide <i>Salmonella</i> prevalence, %	29.9 ^a	82.8 ^b	63.8 ^c	45.2 ^a	9.8 [†]	<0.01
Lymph Nodes, <i>n</i>	44	36	43	38	–	–
Subiliac lymph node <i>Salmonella</i> prevalence, %	11.4	8.3	14.0	0.0	4.7 [†]	0.14

^{a-c}Means within a row with unlike superscripts differ ($p \leq 0.05$).

*Administered sterile saline on day 0 (CON; 5 mL), administered tulathromycin on d 0 (TUL; Draxxin; Zoetis), administered ceftiofur on day 0 (CEF; Excede; Zoetis) or administered florfenicol on day 0 (FLR; Nuflor; Merck Animal Health).

[†]The largest standard error of the mean among the four treatments.

Salmonella hide positives were 33.9% and 18.5% greater for CEF treatment compared to CON and FLR treatments ($p \leq 0.03$) respectively. Lastly, there was only a tendency for FLR treatment to result in a greater prevalence of *Salmonella* compared to the CON ($p = 0.07$). Conversely, no differences were detected among treatments for *Salmonella* prevalence in lymph nodes at study end ($p = 0.14$).

Total, 8ERY^R and 128 ERY^R *Enterococcus* spp. counts from faecal samples

For total *Enterococcus*, a treatment \times day interaction was detected for the concentrations of this bacterial population ($p < 0.01$; Figure 2a). On day 0, the FLR treatment had greater concentrations compared to the CON treatment ($p < 0.01$). Whereas, faecal concentrations of all other treatments did not differ ($p \geq 0.30$), but there was a tendency for FLR to be greater than CEF ($p = 0.08$) and TUL to be greater than CON ($p = 0.08$). On day 28, the CON treatment had greater faecal concentrations compared to both CEF and FLR ($p \leq 0.01$). Additionally, the TUL treatment had counts greater than the FLR treatment group ($p = 0.01$), and there was a tendency for TUL to be greater than CEF ($p = 0.06$). On day 56, there were no differences in concentrations among the treatments ($p \geq 0.16$). On day 112, \log_{10} CFU counts of the TUL treatment were greater compared to FLR treatment ($p = 0.01$), and there was a tendency for CEF treatment to be greater than FLR ($p = 0.10$). On day 182, the CON treatment had fewer counts compared to TUL, CEF and FLR ($p \leq 0.03$). Faecal *Enterococcus* spp. concentrations for TUL, CEF and FLR were not different on day 182 ($p \geq 0.81$). At study end, concentrations for the FLR treatment were lesser than CON, TUL and CEF treatments ($p \leq 0.05$). However, \log_{10} CFU counts were not different among CON, TUL and CEF on the day before harvest ($p \geq 0.27$).

Additionally, trends were observed within treatments across time for total faecal *Enterococcus*. With the exception of FLR, faecal concentrations of total *Enterococcus* increased in CON, TUL and CEF treatments from day 0 to day 28 and from day 182 to study end ($p < 0.05$). Furthermore, the concentration of total *Enterococcus* decreased for all experimental treatment groups from day 28 to day 56 ($p < 0.01$), and then increased again from day 56 to day 112 ($p < 0.01$).

A treatment \times day interaction was detected for the faecal concentrations of erythromycin-resistant (8ERY^R, 8 mg L⁻¹ erythromycin) *Enterococcus* spp. ($p < 0.01$; Figure 2b). On days 0, 56 and 112 there were no differences in the faecal concentrations among metaphylactic treatments ($p \geq 0.13$). Nonetheless, on day 28, both the

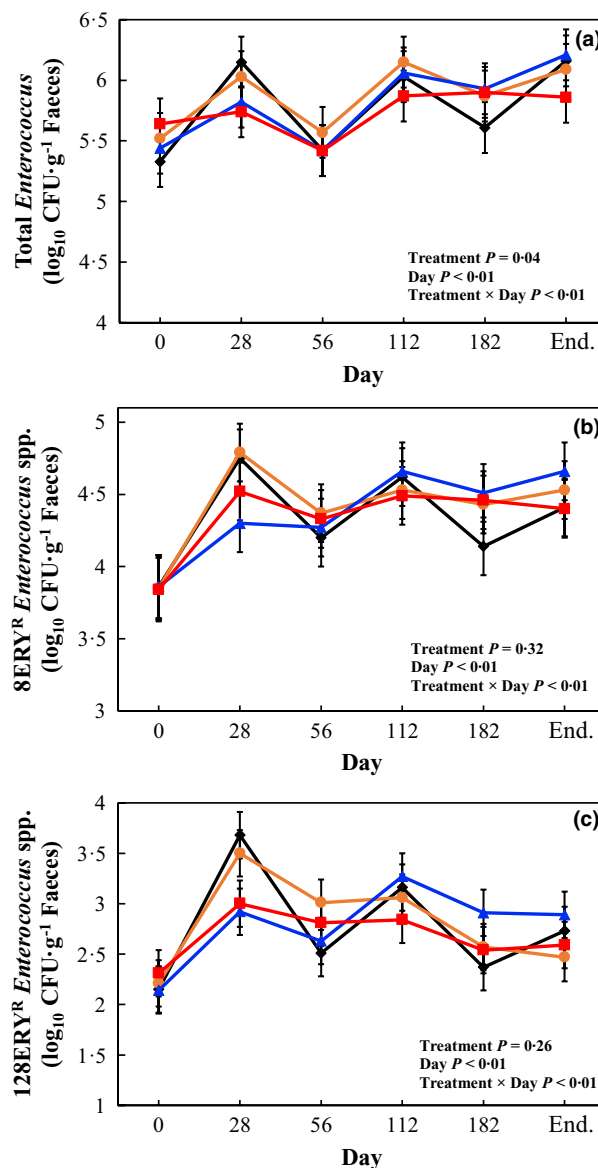


FIGURE 2 (a) Log₁₀ CFU counts g⁻¹ faeces of *Enterococcus* spp. plated on agar without antimicrobial supplementation, (b) Erythromycin resistant (8ERY^R, 8 mg L⁻¹ erythromycin) *Enterococcus* spp., and (c) Erythromycin resistant (128ERY^R, 128 mg L⁻¹ erythromycin) *Enterococcus* spp. in faecal samples collected from cattle given metaphylactic antimicrobial treatments of tulathromycin (Draxxin; orange line; ●), ceftiofur (Excede; blue line; ▲) or florfenicol (Nuflor; red line; ■) compared to cattle not given a metaphylactic antimicrobial (Control; black line; ◆) on day 0. Error bars represent standard error of the mean. Study end was on day 252 for block 1 and day 242 for block 2.

CON and TUL treatments had greater counts than CEF and FLR ($p \leq 0.04$). Additionally, the faecal concentration for FLR was greater than CEF ($p = 0.05$). On day 182, the CON treatment had fewer counts compared to TUL, CEF and FLR treatments ($p \leq 0.01$). Furthermore, faecal concentrations for the TUL, CEF and FLR treatments were not different on day 182 ($p \geq 0.48$). Finally, at study end,

faecal concentrations of this group of antibiotic-resistant *Enterococcus* were greater for the CEF treatment than both the CON and FLR treatments ($p \leq 0.03$).

For the erythromycin highly-resistant (128ERY^R, 128 mg L⁻¹ erythromycin) *Enterococcus* spp. populations, a treatment \times day interaction was detected for mean faecal concentrations ($p < 0.01$; Figure 2C). On day 0, there were no differences in the concentrations among treatments ($p \geq 0.27$). On day 28, the faecal concentrations for both the CON and TUL treatments were greater than CEF and FLR treatments ($p \leq 0.01$). On day 56, TUL had greater counts than both CON and CEF treatments ($p \leq 0.02$), and there was a tendency for FLR treatment to be greater than the CON ($p = 0.07$). On day 112, the FLR treatment had fewer counts compared to the CON and CEF treatments ($p \leq 0.03$). On day 182, faecal concentrations for the CEF treatment were greater than CON, TUL and FLR treatments ($p \leq 0.03$). Furthermore, counts for CON, TUL and FLR treatments did not differ on day 182 ($p \geq 0.25$). Finally, at study end, faecal concentrations for the CEF treatment were greater compared to TUL treatment ($p = 0.01$), and there was a tendency for CEF treatment to be greater than FLR ($p = 0.07$).

Total, TET^R, COT^R, CTX^R and 128ERY^R *Escherichia coli* counts and prevalence from faecal samples

Overall, there was only a tendency for a treatment \times day interaction in mean faecal concentrations for total *E. coli* ($p = 0.10$; Figure 3a). Furthermore, no differences in the counts were detected among treatments ($p = 0.45$). However, differences in faecal concentrations among days were detected ($p < 0.01$). Faecal total *E. coli* counts were fewest among all days on day 0 ($p < 0.01$) and were fewer on day 56 than day 28, 112, 182 or study end ($p \leq 0.01$). Additionally, the total *E. coli* counts were fewer on day 28 than day 112, 182 and study end ($p < 0.01$). Lastly, log₁₀ CFU counts did not differ among each other ($p \geq 0.21$) and were greatest among all days ($p < 0.01$) on days 112, 182 and study end.

A treatment \times day interaction was detected for faecal concentrations of tetracycline resistant (TET^R, 32 mg L⁻¹ tetracycline) *E. coli* ($p < 0.01$; Figure 3b). There were no differences in the counts among treatments on day 56 and 182 ($p \geq 0.12$). On day 0, the concentrations for TET^R *E. coli* for both CEF and FLR treatments were greater than the CON treatment ($p \leq 0.01$), and there was a tendency for TUL treatment to be greater than the CON treatment ($p = 0.09$). On day 28, the faecal concentrations for the FLR treatment were greater than CON and TUL treatments ($p \leq 0.01$), and there was a tendency for FLR to be greater than CEF ($p = 0.09$). Furthermore, on day 28, CEF treatment had

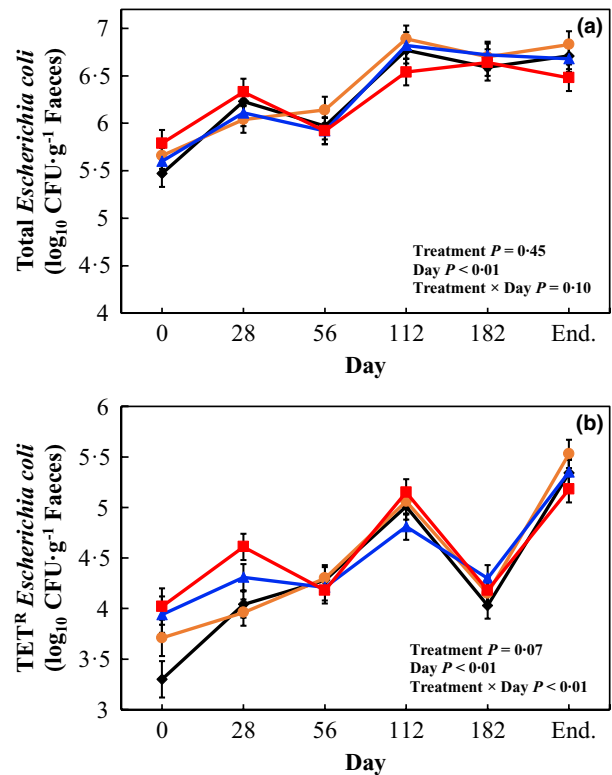


FIGURE 3 (a) Log₁₀ CFU counts g⁻¹ faeces of *Escherichia coli* plated on agar without antimicrobial supplementation and (b) Tetracycline resistant (TETR, 32 mg L⁻¹ tetracycline) *Escherichia coli* in faecal samples collected from cattle given metaphylactic antimicrobial treatments of tulathromycin (Draxxin; orange line; ●), ceftiofur (Excede; blue line; ▲) or florfenicol (Nuflor; red line; ■) compared to cattle not given a metaphylactic antimicrobial (Control; black line; ◆) on day 0. Error bars represent standard error of the mean. Study end was on day 252 for block 1 and day 242 for block 2.

greater counts than TUL treatment ($p = 0.04$). On day 112, FLR treatment had greater mean counts than CEF ($p = 0.05$), while there were no differences among any other treatments ($p \geq 0.16$). Lastly, at study end, mean concentrations were greater for the TUL treatment compared to FLR ($p = 0.05$); however, no differences were detected among any other treatments ($p \geq 0.31$).

For trimethoprim-sulphamethoxazole resistant (COT^R, 76 mg L⁻¹ sulphamethoxazole and 4 mg L⁻¹ trimethoprim) *E. coli* in faeces, a treatment \times day interaction was detected for concentrations ($p < 0.01$; Figure 4a). On day 0, there were no differences in faecal concentrations among treatments ($p \geq 0.74$). However, on day 28, faecal concentrations for FLR treatment were greater than CON, TUL and CEF treatments ($p \leq 0.01$). Additionally, TUL treatment also had fewer counts compared to CON and CEF treatments on day 28 ($p \leq 0.01$). On day 56, faecal concentrations for FLR treatment were greater compared to CON, TUL and CEF treatments ($p \leq 0.01$). Control, TUL and CEF treatments

were not different on day 56 ($p \geq 0.36$). Similarly, on day 112, FLR treatment had greater counts than CON, TUL and CEF treatments ($p \leq 0.05$). Furthermore, faecal concentrations for the CON treatment were greater compared to TUL treatment ($p < 0.01$), and there was a tendency for the CON treatment to be greater than CEF treatment ($p = 0.06$). On day 182, the CON treatment had fewer counts compared to CEF and FLR treatments ($p \leq 0.01$), and there was a tendency for the CON treatment to be less than TUL treatment ($p = 0.09$). Lastly, there was a tendency for faecal concentrations of TUL treatment to be greater than FLR treatment ($p = 0.06$) and a tendency for CEF treatment to be greater than FLR treatment, at study end ($p = 0.10$).

A treatment \times day interaction was detected for percent prevalence of trimethoprim-sulphamethoxazole resistant (COT^R; 76 mg L⁻¹ sulphamethoxazole and 4 mg L⁻¹ trimethoprim) *E. coli* ($p < 0.01$; Figure 4b). There were no

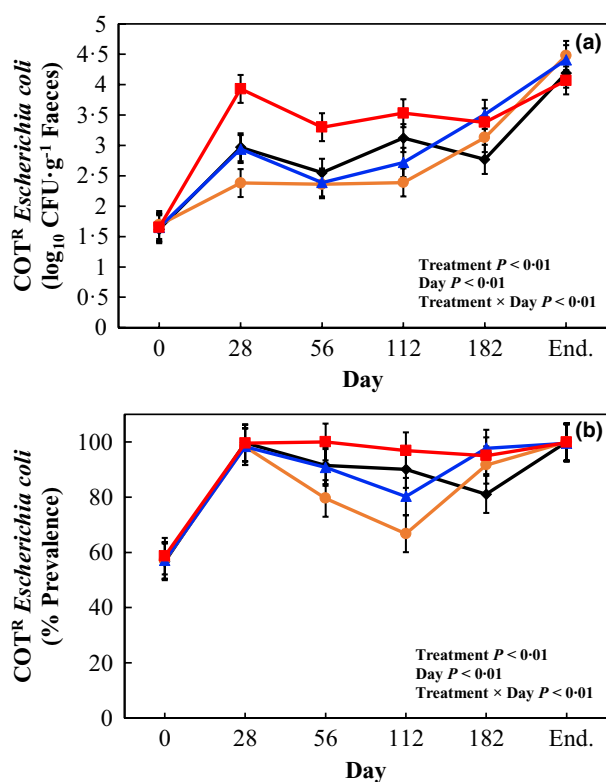


FIGURE 4 (a) Log₁₀ CFU counts g⁻¹ faeces of trimethoprim-sulphamethoxazole resistant (COT^R 31, 76 mg L⁻¹ sulphamethoxazole and 4 mg L⁻¹ trimethoprim) *Escherichia coli* and (b) Percent prevalence of trimethoprim-sulphamethoxazole resistant (COT^R, 76 mg L⁻¹ sulphamethoxazole and 4 mg L⁻¹ trimethoprim) *Escherichia coli* in faecal samples collected from cattle given metaphylactic antimicrobial treatments of tulathromycin (Draxxin; orange line; ●), ceftiofur (Excede; blue line; ▲) or florfenicol (Nuflor; red line; ■) compared to cattle not given a metaphylactic antimicrobial (Control; black line; ◆) on day 0. Error bars represent standard error of the mean. Study end was on day 252 for block 1 and day 242 for block 2.

differences in prevalence among treatments on day 0, 28 and study end ($p \geq 0.70$). On day 56, prevalence of TUL treatment was 11.9, 11.2 and 20.7% less compared to CON, CEF and FLR treatments respectively ($p \leq 0.04$). On day 112, prevalence of TUL treatment was 23.3, 13.5 and 30.1% less compared to CON, CEF and FLR treatments respectively ($p \leq 0.01$). Additionally, FLR treatment had 16.6% greater prevalence than CEF treatment ($p < 0.01$). On day 182, prevalence of the CON treatment was 16.7% less than CEF treatment ($p < 0.01$) and 14.0% less than FLR treatment ($p = 0.01$).

A treatment \times day interaction was detected for mean faecal concentrations of cefotaxime resistant (CTX^R, 2 mg L⁻¹ cefotaxime) *E. coli* ($p < 0.01$; Figure 5a). On day 0, there were no differences in mean faecal counts among treatments ($p \geq 0.35$). On day 28, faecal concentrations for CEF and FLR treatments were greater than the CON and TUL treatments ($p \leq 0.01$). Additionally, there was also a tendency for concentrations for the CON treatment to be greater than TUL treatment on day 28 ($p = 0.07$). On day 56, the TUL treatment had fewer counts compared to CON, CEF and FLR treatments ($p \leq 0.01$). Faecal concentrations for FLR treatment were greater than the CON treatment ($p = 0.01$), and there was a tendency for FLR to be greater than CEF treatment on day 56 ($p = 0.09$). On day 112, the CON treatment had fewer counts compared to TUL and CEF treatments ($p \leq 0.03$), and there was a tendency for the CON treatment to be less than FLR treatment ($p = 0.07$). Additionally, CEF treatment had greater counts compared to FLR treatment ($p = 0.03$), and there was a tendency for CEF treatment to be greater than TUL treatment ($p = 0.06$). On day 182, faecal concentrations for CEF treatment were greater compared to the CON treatment ($p = 0.02$), and there was a tendency for CEF treatment to be greater than FLR treatment ($p = 0.06$). The day before harvest (study end), there was only a tendency for CEF treatment to have greater counts compared to FLR treatment ($p = 0.06$).

A treatment \times day interaction was detected for prevalence of cefotaxime resistant (CTX^R, 2 mg L⁻¹ cefotaxime) *E. coli* ($p < 0.01$; Figure 5b). There were no differences in prevalence among treatments on day 28 and study end ($p \geq 0.62$). On day 0, the CON treatment was 13.3% less than FLR treatment ($p = 0.02$). On day 56, prevalence for TUL treatment was 30.2, 29.5 and 36.8% less than CON, CEF and FLR treatments respectively ($p \leq 0.01$). On day 112, prevalence for CEF treatment was 32.9, 29.4 and 23.1% greater compared to CON, TUL and FLR treatments respectively ($p \leq 0.01$). Similarly, on day 182, CEF treatment was 12.1, 12.3 and 13.7% greater than CON, TUL and FLR treatments respectively ($p \leq 0.04$).

For erythromycin-resistant (ERY^R, 128 mg L⁻¹ erythromycin) *E. coli*, a treatment \times day interaction was detected

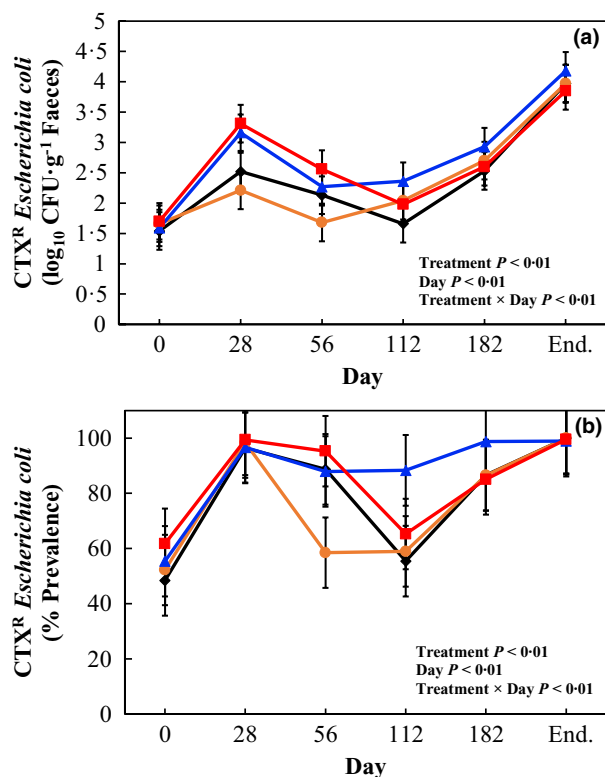


FIGURE 5 (a) Log_{10} CFU counts g^{-1} faeces of third-generation cephalosporin-resistant (CTXR 40, 2 mg L^{-1} cefotaxime) *Escherichia coli* and (b) Percent prevalence of 3rd generation cephalosporin-resistant (CTXR, 2 mg L^{-1} cefotaxime) *Escherichia coli* in faecal samples collected from cattle given metaphylactic antimicrobial treatments of tulathromycin (Draxxin; orange line; ●), ceftiofur (Excede; blue line; ▲) or florfenicol (Nuflor; red line; ■) compared to cattle not given a metaphylactic antimicrobial (Control; black line; ◆) on day 0. Error bars represent standard error of the mean. Study end was on day 252 for block 1 and day 242 for block 2.

for the faecal concentrations ($p < 0.01$; Figure 6). There were no differences in concentrations among treatments on day 0 or 182 ($p \geq 0.43$). On day 28, the counts for FLR treatment were greater than CON, TUL and CEF treatments ($p \leq 0.01$). Furthermore, counts for CEF treatment were greater than CON and TUL treatments on day 28 ($p \leq 0.02$). On day 56, FLR treatment had greater counts compared to CON and TUL treatments ($p \leq 0.02$). On day 112, faecal concentrations for CEF and FLR treatment were greater than the CON and TUL treatments ($p \leq 0.04$). Furthermore, at study end, faecal concentrations for TUL and CEF were greater than the CON and FLR treatments ($p \leq 0.03$).

DISCUSSION

The World Health Organization (WHO) classifies antimicrobials based on their importance to human

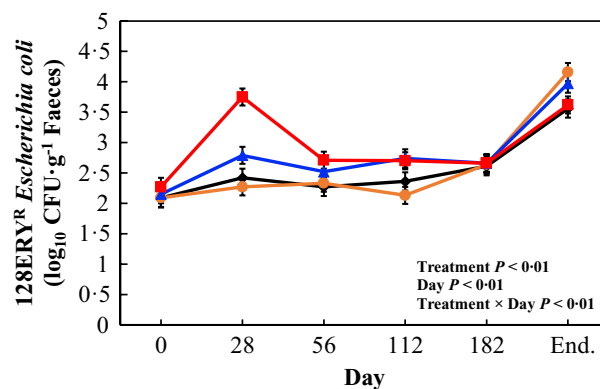


FIGURE 6 Concentration of erythromycin resistant (ERYR, 128 mg L^{-1} erythromycin) *Escherichia coli* in faecal samples collected from cattle given metaphylactic antimicrobial treatments of tulathromycin (Draxxin; orange line; ●), ceftiofur (Excede; blue line; ▲) or florfenicol (Nuflor; red line; ■) compared to cattle not given a metaphylactic antimicrobial (Control; black line; ◆) on day 0. Error bars represent standard error of the mean. Study end was on day 252 for block 1 and day 242 for block 2.

medicine. The WHO has two AMR ranking systems, and antimicrobial classes categorized as both ‘critically important’ and ‘of the highest importance to human medicine’ include cephalosporins, glycopeptides, macrolides, polymyxins and quinolones (WHO, 2019). In this study, we used NARMS antibiotic resistance surveillance recommendations for evaluating antimicrobial resistance targets and concentrations as used in previous studies (Agga et al., 2016). Bacterial resistance to macrolides was evaluated using the antimicrobial erythromycin and bacteria resistance to cephalosporins was evaluated using the third-generation cephalosporin, cefotaxime. Additionally, bacterial resistance to sulphonamides was evaluated using the antimicrobial trimethoprim-sulphamethoxazole and resistance to tetracyclines was also analysed using tetracycline. Results from the current study indicate few contributions of metaphylactic antimicrobial use in beef cattle to AMR of importance to human medicine.

Salmonella

Salmonella is a leading cause of food-borne illness and hospitalizations each year in the United States (Scallan et al., 2011). Commercial abattoirs take numerous precautions to reduce prevalence of *Salmonella* from faecal and hide contamination using techniques such as organic acid rinses, carcass washing and carcass trimming (Galland, 1997). Nonetheless, *Salmonella* can also colonize lymphatic tissue in beef cattle and as such has potential to end up in the ground beef supply via trimmings

(Arthur et al., 2008; Koohmaraie et al., 2012). In the current study, *Salmonella* was detected frequently and there were differences in total prevalence and enumeration of *Salmonella* in this study; however, *Salmonella* resistant to either tetracycline or a third-generation cephalosporins was not detected.

Unlike this study, Levent et al. (2019) reported no difference in faecal prevalence of *Salmonella* among cattle administered tulathromycin or ceftiofur and the control group. However, they did observe a day effect across treatments. Furthermore, they reported prevalence of *Salmonella* in tulathromycin- and ceftiofur-treated cattle was greater at day 56 and remained greater throughout day 112 compared to days 0, 7, 14 and 28. Findings from the current study show a similar trend as *Salmonella* prevalence was greater for control, ceftiofur and tulathromycin treatments from day 112 to study end compared to day 0. Although not measured by Levent et al. (2019), florfenicol followed the same trend in this study with the exception that there was no difference in *Salmonella* prevalence between day 0 and day 182.

The average prevalence of *Salmonella* from hide swabs in the current study was 55.4% (29.9–82.8%) and falls well within the range of averages (15.4–100%) reported from previous research (Bacon, Sofos, Belk, Hyatt, & Smith, 2002; Beach, Murano, & Acuff, 2002; Brichta-Harhay et al., 2011; Fluckey, Loneragan, Warner, & Brashears, 2007; Gragg et al., 2013; Koohmaraie et al., 2012; Levent et al., 2019). Similar to the current study, Levent et al. (2019) measured *Salmonella* prevalence of *Salmonella* from hide swabs and *subiliac* lymph nodes in cattle given a metaphylactic antimicrobial and reported no differences among treatments for hide swabs or lymph nodes. Although not significantly different, Levent et al. (2019) reported the control was 14.6% less than ceftiofur and 9.4% less than tulathromycin. In contrast, the current study detected a difference in hide swabs but no difference in lymph nodes. Treatment groups were collected in the order of control, tulathromycin, ceftiofur and florfenicol suggesting chute order did not confound hide swab results. Furthermore, differences detected in hide swabs were similar to differences in faecal *Salmonella* prevalence at study end with the exception that tulathromycin was not greater than ceftiofur. It should also be noted there was a rainfall event during the final block 1 collection that could have affected hide swab *Salmonella* prevalence.

Numerous studies have reported relatively low prevalence of *Salmonella* isolated from lymph nodes in cattle (Arthur et al., 2008; Bailey et al., 2017; Smith et al., 2021; Webb et al., 2017). Results from the current study support these findings as the average *Salmonella* prevalence isolated from lymph nodes was 8.4% (0.0–14.0%). However, seasonality and location are often implicated to affect

prevalence of *Salmonella*. Webb et al. (2017) indicated a greater prevalence of *Salmonella* in lymph nodes during warmer months for feedlot cattle; although, cattle in the current study were harvested in July and August.

Salmonella, like many Gram-negative bacteria, possesses an intrinsic resistance to certain macrolides because of their inability to penetrate the lipopolysaccharide barrier. However, alteration of macrolide structures has allowed for the development of macrolide antimicrobials such as tulathromycin, a novel triamilide, that are able to mitigate this intrinsic resistance (Evans, 2005; Vaara, 1993). As of 2015, Valenzuela, Sethi, Aulik, and Poulsen (2017) reported no trend for increasing resistance of *Salmonella* to tulathromycin at the minimum inhibitory concentration (MIC) of $32\mu\text{g ml}^{-1}$. However, prevalence and enumeration results from the current study suggest *Salmonella* is increased with the use of tulathromycin. Considering that Gram-negative bacteria have intrinsic resistance to some macrolides, the use of this antimicrobial was not expected to increase *Salmonella*. In veterinary practice, tulathromycin is used primarily for the treatment of BRD. Further research should be conducted to determine if *Salmonella* prevalence is affected by use of tulathromycin.

***Enterococcus* spp.**

Enterococcus spp. were monitored in the current study for potential pathogenic Gram-positive bacteria because it is recognized by NARMS as a broadly distributed indicator to track AMR in Gram-positive species (FDA, 2020). Pathogenic Gram-positive bacteria are of importance because they include foodborne pathogens such as *Listeria monocytogenes*, *Bacillus* spp. and *Clostridium* spp. as well as major bacteria of clinical concern such as *Staphylococcus aureus* (Bintsis, 2017; Gray & Killinger, 1966).

Differences in faecal concentrations of total, 8 mg L^{-1} erythromycin resistant, and 128 mg L^{-1} erythromycin-resistant *Enterococcus* spp. among treatments on specific days in the current study suggest antimicrobial exposure and time alters concentrations of enterococci. Similarly, Vikram et al. (2017) reported exposure to antimicrobials decreased the concentration of generic *Enterococcus* and increased the concentration of erythromycin-resistant *Enterococcus* when compared to cattle raised without antimicrobials. Furthermore, results from Doster et al. (2018) indicated an increase in AMR gene equivalents for tetracyclines and macrolides in control cattle and cattle treated with metaphylactic tulathromycin from days 1 to 11. Similarly, in this study, erythromycin-resistant *Enterococcus* spp. prevalence was greater on day 28 compared to day 0 in both control- and

tulathromycin-treated cattle. In contrast to these results, a study comparing cows treated with ceftiofur to cows not treated with ceftiofur by Agga et al. (2016) reported no differences in \log_{10} CFU counts of nontype-specific enterococci.

Previous research has demonstrated clinical strains of *Enterococcus* spp. to be highly resistant to erythromycin at concentrations of 128 mg L^{-1} (Portillo et al., 2000). In the current study, there is a difference in the concentration for 128 mg L^{-1} erythromycin-resistant *Enterococcus* spp. over time, and these changes mirrored the changes observed with the 8 mg L^{-1} erythromycin-resistant *Enterococcus* spp. with day 28 exhibiting the greatest values. For this study, cattle received an ionophore in the diet. Ionophores are antimicrobials commonly used in cattle production to increase efficiency of growth; however, their mechanism of action is linked to the cell membrane and as such, they are more effective against Gram-positive bacteria (Callaway et al., 2003). Therefore, the inclusion of the ionophore monensin in the diet could have affected the prevalence of *Enterococcus* spp. within the rumen and subsequently the faecal samples. In support, Nisbet, Callaway, Edrington, Anderson, and Poole (2008) reported ionophores significantly decreased the growth rate of *Enterococcus faecium* and *Enterococcus faecalis*. Although not significant, ionophores also decreased the *E. faecalis* population by approximately $0.5 \log_{10}$ CFU mL^{-1} . Further research should be conducted to determine the impact of including monensin in the diet on antimicrobial resistance among bacterial populations.

The minimum temperature during the study was -17.7°C , while the average winter temperatures in Lubbock, TX ranges from -3.3° to -1.1°C . It is likely season affected the faecal concentrations of total *Enterococcus* spp. in this study. Mcauley, Britz, Gobius, and Craven (2015) and Sinton, Braithwaite, Hall, and Mackenzie (2007) both reported increases of *Enterococcus* spp. during spring and summer months isolated from raw milk and faecal pats respectively. In the current study, day 56 of both blocks occurred around a cold-weather event, and as such, likely decreased counts of total *Enterococcus* spp compared to later collection days during warmer seasons.

Furthermore, the increase in total *Enterococcus* spp. on day 28 is likely the result of antimicrobial exposure as control cattle would have also received antimicrobials for treatment more frequently during this portion of the receiving phase. For this study, Coppin (2021) reported some cattle from each experimental treatment group received a follow-up therapeutic antimicrobial treatment; and, as expected, the control group was treated the most therapeutically. Because total *Enterococcus* spp. represent resistant *Enterococcus* spp. populations as well, it is possible the increase in concentration on day 28 is reflective

of therapeutic antimicrobials administered during initial receiving period.

Escherichia coli

Escherichia coli is a major bacterium of concern and food-borne pathogenic *E. coli* cause approximately 344,800 infections each year in the United States. Moreover, it also is used by NARMS as a surrogate species to track AMR within Gram-negative bacteria (CDC, 2018; FDA, 2020).

In the current study, faecal concentrations of total *E. coli* decreased from day 28 to 56 and were greatest on day 112, 182 and the day before harvest. However, metaphylactic treatment did not affect counts of total *E. coli*. A study by Pereira et al. (2020) measuring faecal counts of *E. coli* in dairy calves following administration of an antimicrobial presented similar trends. Pereira et al. (2020) reported a decrease in *E. coli* for all treatment groups on day 56 followed by a peak on day 112. Additionally, there were no significant differences in the average counts of *E. coli* among treatment groups for any of the time points.

Season likely affected faecal concentrations of total *E. coli* in the current study. Stanford, Johnson, Alexander, Mcallister, and Reuter (2016) reported a majority of *E. coli* serogroups collected from cattle faeces were less prevalent during winter. Furthermore, Vikram et al. (2017) also reported season effects on *E. coli* populations in cattle faeces as there was less prevalence of generic, tetracycline resistant, trimethoprim-sulphamethoxazole and third-generation cephalosporin-resistant *E. coli* during winter compared to summer months. In this experiment, a significant cold weather event occurred around day 56 and as a result may have decreased counts of total *E. coli*. These findings support results from the current study as counts of total *E. coli* were greatest on day 112, 182 and study end which occurred during spring and summer.

E. coli resistant to tetracyclines is widespread in animals used for food production (Tadesse et al., 2012). Differences on day 0 may have affected differences on subsequent days; and, with the exception of day 0 and 28, there were no differences in \log_{10} CFU counts of TET^R *E. coli* among the control and any metaphylactic treatment in the current study. Other research supports these results as Mirzaagha et al. (2011) and Vikram et al. (2017) both reported TET^R *E. coli* populations in cattle were not affected by exposure to antimicrobials. In general, the total and the AMR *E. coli* counts increased over time in the current study, and it is also possible other factors contributed to the increase in counts of TET^R *E. coli* between day 0 and study end. For example Berry et al. (2006) observed significantly greater faecal *E. coli* concentrations in cattle fed a grain-based diet compared to cattle fed hay or silage-based diets

and Alexander et al. (2008) observed greater prevalence of TET^R *E. coli* in steers fed a grain-based diet compared to a silage-based diet.

Schmidt et al. (2015) conducted a study measuring antimicrobial-resistant *E. coli* right before and during the processing of feedlot cattle. In that study, faecal prevalence of COT^R *E. coli* was 98.4% when sampled 20–25 days before harvest and 95.1% at processing. In the current study, faecal prevalence of COT^R *E. coli* the day before harvest was similar at 100% (99.5–100%). Furthermore, Schmidt et al. (2015) also measured the concentration of COT^R *E. coli* from faecal samples and reported 94% of values were between 0 and 3.99 log₁₀ CFU per swab at the feedlot and 80.4% of values were between 0 and 2.99 at harvest. However, they also observed concentrations as high as 5.39 and 4.76 log₁₀ CFU per swab at the feedlot and harvest respectively. In the current study, concentration of COT^R *E. coli* the day before harvest was 4.29 (4.07 to 4.48) log₁₀ CFU per gram of faeces.

In this experiment, prevalence and count of COT^R *E. coli* increased for all treatments from days 0 to 28 by 41.6% and 1.4 log₁₀ CFU per g of faeces respectively. Diet and the new pen environment were likely factors in this increase. Cattle in this study were housed in soil pens for the receiving period and previous research has demonstrated that some *E. coli*, like *E. coli* O157:H7, can survive for long periods of time in the faeces of cattle on forage-based rations (Kudva, Blanch, & Hovde, 1998; Wells, Berry, & Varel, 2005). Another possible environmental source of *E. coli* is contaminated water troughs. Lejeune, Besser, and Hancock (2001) reported a decrease over time in *E. coli* isolated from microcosms simulating water troughs. However, *E. coli* was able to survive for 245 day, and microcosm water samples containing *E. coli* were still able to infect young dairy calves up to 183 day later. Therefore, it is possible COT^R *E. coli* persisted in the soil pens and water troughs from previous groups of cattle and spread to the high-risk cattle in the current study at arrival.

Although cattle did not receive a sulphonamide antimicrobial, there were still potential treatment differences in faecal COT^R *E. coli* prevalence and concentrations in this study. After injection of a metaphylactic antimicrobial, the florfenicol treatment had counts of COT^R *E. coli* on days 28, 56 and 112. These results could indicate a cross or co-resistance between florfenicol and trimethoprim-sulphamethoxazole. Co-resistance is the process by which bacteria mutate or acquire mobile genetic elements containing multiple resistance genes to become resistant to different antimicrobials. Whereas cross-resistance is bacteria acquiring resistance to two antimicrobials because they target the same pathway (Cantón & Ruiz-Garbajosa, 2011; Chapman, 2003). Results from Jensen et al. (2018) support findings from this study as they observed *E. coli* resistant to

florfenicol and sulphonamide or trimethoprim. However, it is important to note the study was conducted on swine and the number of *E. coli* isolated resistant to florfenicol was only 4. Although of those 4, 100% were resistant to sulphonamides and 50% were resistant to trimethoprim. There is a lack of literature on cross-resistance and co-resistance in cattle receiving antimicrobials on the feedlot and further research should be conducted on the subject.

Injection of ceftiofur resulted in greater counts and concentrations of CTX^R *E. coli* at certain time points in this study. In contrast to these results, Agga et al. (2016) reported no effect of ceftiofur injection on CTX^R *E. coli* prevalence in beef cows. Furthermore, Taylor et al. (2019) reported the faecal counts of third-generation cephalosporin *E. coli* returned to pretreatment counts by day 28 in dairy cows receiving 2 doses of ceftiofur. In addition, Kanwar et al. (2013) reported an initial increase in prevalence of cephalosporin-resistant *E. coli* in steers administered ceftiofur, but prevalence returned to basal values by day 26. In the current study, a decrease towards initial faecal counts of CTX^R *E. coli* did not occur until day 56 and prevalence of CTX^R *E. coli* remained high after the initial increase on day 28. Schmidt, Griffin, Kuehn, and Brichta-Harhay (2013) reported an 83.8% increase in cephalosporin-resistant *E. coli* 3 to 8 day following therapeutic injection of ceftiofur in steers which was maintained throughout the period of increased disease susceptibility. In the current study, prevalence of CTX^R *E. coli* increased 43.3% from days 0 to 28 and high prevalence was maintained throughout the study for the ceftiofur treatment. The increase in prevalence and counts of CTX^R *E. coli* beginning on day 112 are likely a result of factors discussed previously such as diet, environment and season.

Many Gram-negative bacteria like *E. coli* are naturally resistant to low concentrations (<64 mg L⁻¹) of erythromycin, but some clinical *E. coli* strains might be susceptible to higher concentrations (>64 mg L⁻¹; Nguyen et al., 2009). More important, those strains exhibiting resistance to high concentrations of erythromycin were potentially multi-drug resistant. Results from this study suggest tulathromycin and ceftiofur increase faecal counts of 128ERY^R *E. coli* at the end of the finishing period. Foditsch, Pereira, Siler, Altier, and Warnick (2019) conducted a faecal microbiome analysis of heifer calves administered tulathromycin and reported no differences in erythromycin-resistant populations between control and tulathromycin treatments up to the study conclusion at day 112. This was similar to the trend in the current study, but an increase occurred on day 112 which could be attributed to season as previously discussed. Additionally, this study reported greater counts of 128ERY^R *E. coli* in florfenicol and ceftiofur treatments on day 28. Ma et al. (2014) observed

cross-resistance between amphenicols and erythromycin in *Campylobacter jejuni* caused by a novel point mutation which could explain the day 28 increase in the florfenicol treatment in the current study. Literature analysing the effects of antimicrobial exposure on erythromycin resistant *E. coli* in cattle is limited and further research should be conducted on the subject.

Results from this study indicate faecal concentrations and prevalence for *Salmonella*, *E. coli*, *Enterococcus* spp. and associated antimicrobial resistance populations increase towards the end of the feeding period with the exception of 128 mg L⁻¹ erythromycin-resistant *Enterococcus* spp. Apart from 128ERY^R *E. coli*, *Salmonella* and 8ERY *Enterococcus* spp., injection of a metaphylactic antimicrobial at feedlot arrival did not influence antimicrobial resistance by the end of the feeding period when compared to control cattle. Additionally, antimicrobial administration did not result in detectable strains of *Salmonella* exhibiting AMR. However, prevalence and faecal counts of total *Salmonella* from faecal samples were greater in the tulathromycin treatment compared to the control at study end. Furthermore, prevalence of *Salmonella* from hide swabs was greater for tulathromycin and ceftiofur compared to the control, but there were no treatment differences found for *Salmonella* prevalence in lymph nodes. These results suggest *Salmonella* may be influenced by extrinsic factors with elevated concentrations resulting from the use of tulathromycin when compared to other antimicrobials commonly used for beef cattle. Although tulathromycin resulted in greater *Salmonella* in faecal and hide swab samples, it is not likely to contaminate the human food supply because there was a low prevalence in lymph nodes and multiple measures are taken by harvest facilities to mitigate possible contamination from faeces and hides. In conclusion, antimicrobial resistance in feedlot cattle is not caused solely by using a metaphylactic antimicrobial on arrival, but likely a multitude of environmental and management factors.

AUTHORS' CONTRIBUTION

K.E. Hales, J.E. Wells and S.C. Fernando carried out funding acquisition. N.S. Long and C.M. Bacon carried out project administration. J.E. Wells, E.D. Berry, S.C. Fernando, G.H. Loneragen, D.R. Woerner, P.R. Broadway, J.A. Carroll, N.C. Burdick Sanchez, J.F. Legako, C.M. Bacon, C.L. Helmuth, T.M. Smock, J.L. Manahan, A.A. Hoffman and K.E. Hales carried out project execution. K.E. Hales, J.E. Wells and P.R. Broadway were involved in supervision. N.S. Long, K.E. Hales and J.E. Wells contributed to writing—original draft. J.E. Wells, E.D. Berry, S.C. Fernando, G. H. Loneragen, D.R. Woerner, P.R. Broadway, J.A. Carroll, N.C. Burdick Sanchez, J.F.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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