***Salmonella enterica* and extended-spectrum cephalosporin-resistant *Escherichia coli* recovered from Holstein dairy calves from 8 farms in New Brunswick, Canada**

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ABSTRACT

This study was carried out to determine the frequency of fecal carriage, antimicrobial susceptibility, and resistance genes in *Salmonella enterica* and *Escherichia coli* with reduced susceptibility to extended-spectrum cephalosporins (ESC) isolated from 488 dairy calves from 8 farms in New Brunswick, Canada. Both *S. enterica* and *E. coli* with reduced susceptibility to ESC were isolated using selective culture. Minimum inhibitory concentrations to a panel of antimicrobial drugs were determined for randomly selected *E. coli* isolates and all of the *Salmonella* isolates. Multiplex PCR were conducted on the selected ESC-resistant *E. coli* to assess the β -lactamase resistance genes (*bla*_{CTX-M}, *bla*_{CMY-2}, *bla*_{SHV}, and *bla*_{TEM}) and plasmid-mediated *qnrB* and *qnrS* resistant genes. Information on ceftiofur use and other farm management practices were collected by the use of a questionnaire to determine the risk factors for the fecal recovery of *E. coli* with reduced susceptibility to ESC. *Salmonella enterica* frequency in calves' fecal samples was 3.3%, and all were pansusceptible. *Salmonella* isolates belonged to 3 serovars namely *Salmonella* Senftenberg, *Salmonella* Typhimurium, and *Salmonella* Derby. The frequency of fecal carriage of *E. coli* with reduced susceptibility to ESC in calves was 81.2%. Of the selected isolates ($n = 100$), all were multi-drug resistant, whereas 88% were ESC resistant based on minimum inhibitory concentration testing. From the selected ESC-resistant *E. coli* isolates, *bla*_{TEM} was detected in 84.1%, *bla*_{CMY-2} was detected in 52.2%, *bla*_{CTXM} groups were detected in 30.7%, and *bla*_{SHV} was detected in 1.1% of isolates. Plasmid-mediated quinolone resistance genes were identified in 7 of 9 isolates resistant to quinolones. Five isolates were positive for

qnrB, whereas 2 isolates were positive for both *qnrB* and *qnrS*. Whereas neonatal calves [odds ratio (OR) = 2.42, 95% confidence interval (CI): 1.87–3.12], regular ceftiofur use on the farm (OR = 3.83, 95% CI: 2.29–6.39), feeding of unpasteurized nonsalable milk (OR = 1.6, 95% CI: 1.18–2.18), and use of florfenicol (OR = 2.02, 95% CI: 1.43–2.86) were statistically associated with fecal recovery of *E. coli* with reduced susceptibility to ESC, use of ceftiofur for the treatment of respiratory diseases (OR = 0.57, 95% CI: 0.41–0.79) was statistically associated with decreased recovery of *E. coli* with reduced susceptibility to ESC. This study has provided information on the resistance genes associated with the occurrence of ESC and fluoroquinolone resistance in dairy calves within this region.

Key words: extended-spectrum cephalosporin, fecal shedding, *Enterobacteriaceae*, dairy calf

INTRODUCTION

Antimicrobial resistance (AMR) is a global concern in food animal production systems where AMR pathogens can contribute to increased morbidity and mortality of animals as well as public health concerns (van den Bogaard and Stobberingh, 2000; Call et al., 2008). In dairy calves, mortality and treatment costs are important causes of economic loss to the dairy industry (Donaldson et al., 2006). Various studies have shown that respiratory diseases and diarrhea are the most prevalent causes of death in calves from birth to weaning with *Escherichia coli* and *Salmonella enterica* being 2 of the major bacterial causes of diarrhea-associated mortality (Constable, 2004; Donaldson et al., 2006; Megersa et al., 2009).

Generic *E. coli* are abundant commensal enteric bacteria in animals and humans and are ubiquitous in the environment. They are commonly used as indicator bacteria for monitoring AMR dynamics, especially to very important antimicrobials such as ESC and fluoro-

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quinolones (DeFrancesco et al., 2004; Wang et al., 2006). Antimicrobial susceptibility patterns of *E. coli* reflect the selective pressure exerted by antimicrobial use and the potential of these bacteria to serve as a reservoir of AMR genes (European Food Safety Authority, 2012). These AMR genes, commonly found on plasmids, can be shared between bacteria, including *Salmonella* spp., in both humans and animals (Fluckey et al., 2007). *Salmonella enterica* is an important foodborne pathogen accounting for millions of cases of human infections worldwide (Public Health Agency of Canada, 2014). Salmonellosis is of clinical and production importance in both dairy and beef cattle operations causing illness and economic losses (Edrington et al., 2004). *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Kentucky, *Salmonella* Montevideo, *Salmonella* Dublin, *Salmonella* Heidelberg, and *Salmonella* Newport are some of the major serovars associated with clinical infections in both human and cattle (Alexander et al., 2009; Jackson et al., 2013).

In Canada, extended-spectrum cephalosporins (ESC) are commonly used in both human and veterinary medicine. Although ceftiofur is recommended for undifferentiated bacterial pneumonia and acute bovine interdigital necrobacillosis, extra-label use for the treatment of gastroenteritis in dairy calves is also common (Canadian Veterinary Medical Association, 2008). According to the World Health Organization and Health Canada, ESC are considered to be critically important with respect to human medicine and are frequently prescribed for the treatment of invasive and life-threatening enteric infections including those caused by *Escherichia coli* and *Salmonella enterica* in humans (WHO, 2007; Veterinary Drugs Directorate, Health Canada, 2009). In dairy cattle, increased ESC resistance (ESC-R) has been widely reported in *E. coli* and *S. enterica* and is caused mainly by extended-spectrum β -lactamases (ESBL) or AmpC-type β -lactamases. Additionally, these organisms are commonly multi-drug resistant (MDR; Sawant et al., 2007; Daniels et al., 2009; Seiffert et al., 2013). The emergence of these MDR strains poses an increasing threat to the successful disease management in dairy calves (de Verdier et al., 2012). Dairy calves commonly harbor MDR bacteria with fecal shedding being most prevalent in the first few weeks of life (Berge et al., 2006a). Different studies have linked the fecal shedding of MDR *E. coli* and *S. enterica* to several factors including antimicrobial use (Berge et al., 2005; Daniels et al., 2009), age of calf (Hoyle et al., 2004; Berge et al., 2006a), type of housing (Pereira et al., 2014a), diets and feeding practices (Edrington et al., 2012), nonsalable milk feeding to dairy calves (Brunton et al., 2014; Randall et al., 2014; Duse et al., 2015) as well as long-term maintenance and occurrence

of MDR *E. coli* in the absence of antimicrobial selection (Khachatryan et al., 2004).

Studies have examined and reported on the ESC resistance dynamics in dairy cattle within North America (Winokur et al., 2001; Heider et al., 2009a,b; Mollenkopf et al., 2012; Cormier et al., 2015). The ESC resistance has been predominantly attributed to the cephamycinase gene *bla*_{CMY-2} and to a lesser extent to the ESBL resistance genes such as *bla*_{CTX-M}, *bla*_{SHV}, and *bla*_{TEM} (Allen and Poppe, 2002; Mollenkopf et al., 2012; Schmidt et al., 2013).

Extended-spectrum β -lactamase producing *Enterobacteriaceae*, especially those with *bla*_{CTX-M-15}, *bla*_{SHV-5}, and *bla*_{SHV-7}, have been associated with plasmid-mediated quinolone resistance (PMQR) genes (Nordmann, 2005). In most cases, both groups of genes (ESC and PMQR) are co-located on the same plasmid (Robicsek et al., 2006). However, limited reports are available of detection of PMQR genes in isolates from dairy cattle. Plasmid-mediated quinolone resistance genes mediated by *qnrS1* have been reported from an *E. coli* isolate from cattle in the United Kingdom (Kirchner et al., 2011). Other studies from the Netherlands and the United States have reported *qnrB19* associated with *E. coli* from a veal calf (Hordijk et al., 2011) and 2 *Salmonella* isolates from environmental samples from a dairy farm (Cummings et al., 2017). Except for a study on *E. coli* mastitis on dairy farms (Saini et al., 2013), information on the fecal prevalence, risk factors associated with shedding, and the genetic mechanisms of resistance to ESC and quinolones of *Salmonella enterica* and ESC-R *E. coli* in dairy cattle in Atlantic Canada is limited.

The primary objectives of this study were to determine the frequency of fecal carriage and antimicrobial susceptibility patterns in *S. enterica* and ESC-R *E. coli* from Holstein dairy calves on 8 dairy farms in New Brunswick, Canada, using selective culture methods. Also, to determine the β -lactamase resistance genes associated with *S. enterica* and ESC-R *E. coli*. In addition, we explored the MDR in *S. enterica* and ESC-R *E. coli*, and when these MDR isolates had quinolone resistance, we examined if they harbored PMQR genes. Lastly, we determined the risk factors for fecal recoveries of *Salmonella enterica* and *Escherichia coli* with reduced susceptibility to ESC from Holstein dairy calves from these 8 farms in New Brunswick, Canada.

MATERIALS AND METHODS

Sample Collection

A convenience sample of 8 dairy farms from New Brunswick was recruited. Farms were visited on a fortnightly basis by their regular herd veterinarians

to collect rectal fecal swabs from Holstein dairy calves from July 2014 to February 2015. The sample collection included 488 dairy calves born within this period. These calves were followed from the neonatal period to weaning (removal of all milk in the calves' diet). Rectal fecal swabs were collected using ready to use swab sticks (BBL CultureSwab collection and transport system, Becton and Dickinson and Company, Franklin Lakes, NJ) from 488 calves at 2 time points: 2 to 15 d old (neonatal) and 42 to 56 d old (weaning). Rectal swabs samples were shipped on ice, via courier, to the laboratory following every collection. Samples were processed the same day they were received in the laboratory.

Isolation and Identification of *Salmonella enterica*

Fecal swabs were pre-enriched in 10 mL of buffered peptone water (BPW) and then incubated at 35°C for 18 to 24 h. Aliquots (200 µL) of incubated pre-enrichment broth were struck on modified semi-solid Rappaport-Vassiliadis agar plates (Becton Dickinson and Co., Franklin Lakes, NJ). The plates were incubated at 42°C and then examined for migration pattern typical of *S. enterica* (≥ 20 mm migration) every 24 h for a total of 72 h. Presumptive *Salmonella* isolates were sub-cultured onto MacConkey agar, and 3 to 5 nonlactose-fermenting colonies were purified by sub-culturing onto tryptic soy agar supplemented with 5% sheep blood. *Salmonella enterica* were further identified by triple sugar iron test, utilization of citrate, urease production, and *Salmonella* O antiserum Poly A-I & Vi. All isolates were frozen in Brucella broth with 15% glycerol at -80°C . *Salmonella* serotyping was carried out by the OIE Salmonella Reference Laboratory, National Microbiology Laboratory, Public Health Agency of Canada, Guelph, Ontario.

Isolation and Identification of Suspected Extended-Spectrum Cephalosporin-Resistant *E. coli*

The initial enrichment process for the isolation of *E. coli* was carried out following methods used by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (Government of Canada, 2010). In brief, an initial pre-enrichment was carried out by incubating the fecal swab in 10 mL of BPW overnight at 35°C for 18 to 24 h. Selective enrichment was carried out by adding an equal volume (10 mL) of the incubated BPW to double-strength *Escherichia coli* broth and incubated at 45°C for 18 to 24 h. A tryptic soy agar plate with 5% sheep blood containing vancomycin (6 µg/mL), ceftazidime (2 µg/mL), amphotericin

B (2 µg/mL), and clindamycin (1 µg/mL; VACC) was used for selective culture of *E. coli* with reduced susceptibility to ESC [suspected extended-spectrum cephalosporin-resistant (sESC-R) *E. coli*; Singh et al., 2012]. The VACC agar was inoculated with 50 µL of inoculated *E. coli* broth and incubated at 37°C for 18 to 24 h. If no presumptive colonies grew, then the VACC agar plates were incubated for an additional 18 to 24 h. Suspected ESC-R *E. coli* isolates (3–5 isolates per plate depending on colony morphology) were sub-cultured to eosin methylene blue agar, and typical colony types were purified on tryptic soy agar supplemented with 5% sheep blood. *Escherichia coli* were identified by lactose-fermentation, inability to use citrate, and lack of urease production. All the isolates were frozen in Brucella broth with 15% glycerol at -80°C for further laboratory analysis.

Antimicrobial Susceptibility Testing

From all the sESC-R *E. coli* isolates that grew on the VACC agar, a subset of 100 isolates was randomly selected with at least 8 isolates per farm being selected for antimicrobial susceptibility testing. All of the recovered *Salmonella* isolates were included for antimicrobial susceptibility testing. Broth microdilution using the Sensititer system was used to determine MIC of the isolates. This method conformed to the Clinical and Laboratory Standards Institute standards (CLSI, 2015) and protocols developed by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (Government of Canada, 2010). The CMV2AGNF (Sensititer, Trek Diagnostic Systems, Westlake, OH) gram-negative panel for the National Antimicrobial Monitoring System containing 15 antimicrobials was used in this study. The following antimicrobial agents were tested with the resistance breakpoints presented in parentheses: ampicillin (≥ 32 µg/mL), amoxicillin-clavulanic acid ($\geq 32/16$ µg/mL), chloramphenicol (≥ 32 µg/mL), ceftriaxone (≥ 4 µg/mL), ceftiofur (≥ 8 µg/mL), ciprofloxacin (≥ 1 µg/mL), ceftiofur (≥ 8 µg/mL), ciprofloxacin (≥ 1 µg/mL), cefoxitin (≥ 32 µg/mL), gentamicin (≥ 16 µg/mL), kanamycin (≥ 64 µg/mL), nalidixic acid (≥ 32 µg/mL), streptomycin (≥ 64 µg/mL), sulfisoxazole (≥ 512 µg/mL), trimethoprim-sulfamethoxazole ($\geq 4/76$ µg/mL), and tetracycline (≥ 16 µg/mL). The resistance breakpoints used for these antimicrobials were based on CLSI guidelines (CLSI, 2015). Azithromycin was on the panel but was excluded from analysis because of the intrinsic resistance of *E. coli* to the antimicrobial.

The ESC-R *E. coli* was defined as a sESC-R *E. coli* that showed MIC resistance to either ceftriaxone or ceftiofur, or both, based on their MIC breakpoints. Multi-drug resistance was defined as resistance to at

least one antimicrobial in at least 3 or more antimicrobial categories, as described by the World Health Organization (Magiorakos et al., 2012).

β-Lactamase and Plasmid-Mediated Quinolone Resistance Gene Detection

β-Lactamase gene detection was carried out on all the isolates selected for antimicrobial susceptibility testing. Genomic DNA was extracted using a commercial kit (InstaGene Matrix Bio-Rad, Montreal, Canada). Two multiplex PCR were performed for the molecular detection of β-lactamase genes. The first multiplex PCR for the detection of *bla*_{CMY-2}, *bla*_{SHV}, and *bla*_{TEM} was carried out using the consensus primers and amplification protocol that was previously described (Kozak et al., 2009). The second multiplex PCR was carried out to determine the different *bla*_{CTX-M} groups (group 1, 2, and 9) using consensus primers and the amplification protocol previously described (Dallenne et al., 2010). All cultured isolates exhibiting quinolone resistance by MIC were further characterized for the PMQR genes: *qnrB* group and *qnrS* group genes. This characterization was done using the multiplex PCR protocol and primers described by Cattoir et al. (2007).

For all multiplex PCR, a Qiagen Multiplex PCR Master Mix Kit (Qiagen, Mississauga, Ontario, Canada) with 1× Qiagen multiplex PCR master mixture, 1× Q-solution, and molecular grade water, 1× consensus primer pair mixture, as well as 2 μL of the isolate's DNA template were included in the final 25-μL mixture according to the manufacturer's instructions. Amplification was carried out in a PCR thermocycler (Techne TC-412, Thermo Fisher Scientific, Mississauga, Ontario, Canada). Both positive and negative controls were included in every multiplex PCR. Fragments of DNA were analyzed by electrophoresis on a 1.0% agarose gel at 100 V for 1 h in 1× Tris-boric acid-EDTA containing 1 μg/mL of ethidium bromide.

Risk Factor Analysis: Descriptive, Unconditional, and Multivariable Models

Data management was done using Microsoft Excel for Windows (2010, Microsoft Corp., Redmond, WA) and then imported to STATA/IC 14.0 (StataCorp, College Station, TX) for analysis. Risk factors for the fecal recovery of *S. enterica* and sESC-R *E. coli* were determined using the information from a questionnaire. Information collected through the questionnaire was number of cows and preweaned calves within the herd; breed of cattle; cow and calf housing types; feeding practices of

unpasteurized nonsalable milk to calves; ceftiofur use, frequency of use and what disease conditions ceftiofur was used for; previous history of salmonellosis on the farm; and information on other antimicrobials used on the farm. The questionnaires were mailed to the herd veterinarians who provided them to the enrolled farms; the producers completed the questionnaire and returned to the herd veterinarians who forwarded them to the researchers. For risk factor analysis, all the predictors assessed except calf age at sampling were at the farm level. Initial descriptive statistics were done to summarize the various predictors of interest as well as the outcomes of interest. Collinearity between the variables was assessed using the variance-covariance matrix estimates and Spearman correlations. For highly correlated variables, one of the variables was selected to be included in the subsequent modeling process.

The data structure in this study was a 3-level hierarchical structure with the farm at the highest level, calf as the second level, and the repeated sampling time was the lowest level. Univariable logistic regression models were carried out to determine the candidate variables for multivariable models. Predictors that were significant at *P*-values ≤ 0.20 in the univariate analysis were included in the subsequent multivariable regression model. We first used this data structure to set up a 3-level mixed effects model to examine if the predictors accounted for the farm level variance. However, in the mixed effect model, after accounting for the predictors, the variability between farms disappeared, with all the variation occurring at the calf level. Therefore, calf level hierarchy was used in the subsequent multivariable model. Given that there were only 2 observations per calf, a generalized estimating equation model was initially compared with multivariable logistic regression. Due to the similarity in these models, a population average multivariable logistic regression model (adjusting for clustering at calf level) was subsequently fitted for each outcome of interest. Then the model assessment was carried out by checking the residuals, identifying influential observations, and evaluating the reliability of the model (Dohoo et al., 2010). Confounding and interaction between the variables were examined based on the use of a causal diagram. The goodness of fit test of the model was assessed using either Pearson or Hosmer-Lemeshow tests. The Wald test was used to test for the significance of predictors. Predictors with *P*-values ≤ 0.05 were retained in the final model. Odds ratios were computed and interpreted as a population average. Odds ratio < 1 between a variable and the outcome was considered protective, whereas odds ratio > 1 was considered a risk factor.

RESULTS

Frequency of Fecal Carriage and MIC Profile of *Salmonella enterica* Isolated from Dairy Calves

Sixteen (16) of the 488 calves (3.3%) were shedding *Salmonella enterica* on at least 1 of the 2 sample times. All *S. enterica* isolates were from 4 of the 8 farms (50%), and the within-herd frequency on the 4 positive farms ranged from 2.1 to 6.3%. The *Salmonella* isolates belonged to 3 serovars, namely *Salmonella* Senftenberg, *Salmonella* Typhimurium DT02, and *Salmonella* Derby. All *Salmonella* serovars clustered on farms with *Salmonella* Senftenberg isolated from 2 farms, whereas *Salmonella* Typhimurium and *Salmonella* Derby were found on one farm each. Shedding was more likely to occur during at the neonatal period (3.3%) compared with the weaning period (0.82%; $P = 0.0067$). All 4 calves that were positive at weaning were also positive during the neonatal period. *Salmonella enterica* isolates were pansusceptible to all antimicrobials on the MIC panel except one isolate that was resistant to sulfisoxazole.

Frequency of Fecal Carriage of sESC-R *E. coli* from Dairy Calves and MIC Profiles of Selected sESC-R *E. coli*

Escherichia coli with sESC-R, based on selective culture media, were isolated from 396 of 488 calves (81.2%) over the study period. Fifty-two percent (205 calves) of calves positive for sESC-R *E. coli* were positive at both the neonatal and weaning periods. The within-herd frequency of shedding of sESC-R *E. coli* ranged from 75 to 98.4%. Significant differences ($P < 0.0001$) were observed in the frequency of recovery of sESC-R *E. coli* between the neonatal (71.1%) and weaning (51.1%) periods. From the 100 selected sESC-R *E. coli* isolates for MIC testing (Table 1), resistance was commonly observed to ampicillin (100%), tetracycline (94%), sulfisoxazole (94%), amoxicillin/clavulanic acid (89%), streptomycin (100%), ceftriaxone (88%), cefoxitin (84%), and ceftiofur (85%). Nine percent of sESC-R *E. coli* isolates were nalidixic acid resistant and also showed intermediate susceptibility to ciprofloxacin. Based on MIC testing, 88% of the selected sESC-R *E. coli* were confirmed ESC-R and were all MDR (Table 2).

Frequency of β -Lactamase Resistance Genes in the Selected sESC-R *E. coli*

All 100 sESC-R *E. coli* isolates were examined for the presence of ESC resistance genes. In the 88 *E. coli* isolates confirmed to be ESC-R based on MIC testing,

Table 1. Distribution of MIC for suspected extended-spectrum cephalosporin-resistant *Escherichia coli* ($n = 100$) recovered from fecal samples of dairy calves from New Brunswick, Canada (2014–2015)

Antimicrobial	Range	MIC ₅₀	MIC ₉₀	% R	Distribution (%) of MIC ¹ (μg/mL)													
					0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128
Amoxicillin/clavulanate	1–32	>32	>32	89.0									8.0	3.0	10.0	79.0		
Ampicillin	1–32	>32	>32	100.0												100.0		
Cefoxitin	0.5–32	>32	>32	84.0							3.0	6.0	3.0	4.0	8.0	76.0		
Ceftiofur ²	0.12–8	>8	>8	85.0						6.0	5.0	4.0	26.0	59.0				
Ceftriaxone	0.25–64	16	64	88.0						12.0		3.0	21.0	32.0	19.0	5.0	8.0	
Chloramphenicol	2–32	>32	>32	61.0								8.0	31.0			61.0		
Ciprofloxacin	0.015–4	≤0.015	0.03	0.0	89.0	2.0		9.0										
Gentamicin	0.25–16	1	4	9.0					31.0	49.0	9.0	1.0	1.0	8.0				
Kanamycin	8–64	16	>64	52.0									48.0			1.0	51.0	
Nalidixic acid	0.5–32	2	4	9.0												9.0		
Streptomycin ³	32–64	>64	>64	88.0						11.0	75.0	5.0						
Sulfisoxazole	16–512	>256	>256	94.0										6.0	12.0	4.0	84.0	94.0
Tetracycline	4–32	>32	>32	94.0											5.0	89.0		
Trimethoprim/ sulfamethoxazole	0.12–4	>4	>4	72.0	17.0	9.0	2.0				6.0		72.0					

¹MIC₅₀ = MIC which inhibits visible growth of 50% of the isolates tested; MIC₉₀ = MIC which inhibits visible growth of 90% of the isolates tested; % R = percentage resistant.

²CLSI M31–A2 (CLSI, 2002).

³No Clinical and Laboratory Standards Institute (CLSI) interpretive criteria for *Enterobacteriaceae* were available; the breakpoint was based on the Canadian Integrated Program for Antimicrobial Resistance Surveillance report (Government of Canada, 2010).

Table 2. Multi-drug resistance patterns of selected 88 extended-spectrum cephalosporin-resistant *Escherichia coli* isolated from Holstein dairy calves (2014–2015)¹

No.	Multi-drug resistance pattern	Frequency	%
1	A-Ag-C-Cax-Cef-Fox-Kan-Str-Sul-Tms-Tet	22	25.00
2	A-Ag-C-Cax-Cef-Fox-Str-Sul-Tms-Tet	13	14.77
3	A-Ag-Cax-Cef-Fox-Str-Sul-Tms-Tet	13	14.77
4	A-Ag-Cax-Cef-Fox-Nal-Str-Sul-Tms-Tet	7	7.95
5	A-C-Cax-Cef-Kan-Str-Sul-Tms-Tet	6	6.82
6	A-Ag-C-Cax-Cef-Fox-Gm-Str-Sul-Tms-Tet	4	4.55
7	A-Ag-C-Cax-Cef-Fox-Kan-Str-Sul-Tet	4	4.55
8	A-Ag-C-Cax-Cef-Fox-Str-Sul-Tet	3	3.41
9	A-Ag-C-Cax-Kan-Str-Sul-Tet	2	2.27
10	A-Ag-Cax-Cef-Fox-Str	2	2.27
11	A-Ag-C-Cax-Cef-Fox-Kan-Nal-Str-Sul-Tms-Tet	1	1.14
12	A-Ag-C-Cax-Cef-Gm-Kan-Str-Sul-Tet	1	1.14
13	A-Ag-C-Cax-Cef-Kan-Str-Sul-Tet	1	1.14
14	A-Ag-C-Cax-Cef-Kan-Str-Sul-Tms-Tet	1	1.14
15	A-Ag-Cax-Cef-Fox-Gm-Str-Sul-Tet	1	1.14
16	A-Ag-Cax-Cef-Fox-Kan-Nal-Str-Sul-Tms-Tet	1	1.14
17	A-Ag-Cax-Cef-Fox-Str-Tet	1	1.14
18	A-Ag-Cax-Cef-Gm-Str-Sul-Tms-Tet	1	1.14
19	A-C-Cax-Cef-Gm-Str-Sul-Tms-Tet	1	1.14
20	A-Cax-Cef-Gm-Kan-Str-Sul	1	1.14
21	A-Cax-Cef-Gm-Str	1	1.14
22	A-Cax-Cef-Str	1	1.14

¹A = ampicillin; Ag = amoxicillin-clavulanate; C = chloramphenicol; Cax = ceftriaxone; Cef = ceftiofur; Fox = cefoxitin; Gm = gentamicin; Kan = kanamycin; Nal = nalidixic acid; Str = streptomycin; Sul = sulfisoxazole; Tms = trimethoprim-sulfamethoxazole; Tet = tetracycline.

*bla*_{TEM} was detected in 84.1%, *bla*_{CMY-2} was detected in 52.2%, *bla*_{CTXM} groups were detected in 30.7%, and *bla*_{SHV} was detected in 1.1% of isolates. Of the *bla*_{CTXM}-producing isolates, the frequency of detection of the phylogenetic groups was 9.1% *bla*_{CTXM-1}, 9.1% *bla*_{CTXM-2}, and 15.9% *bla*_{CTXM-9} for all ESC-R *E. coli*. Forty-eight percent (48%) of ESC-R *E. coli* had 2 β -lactamase resistance genes, and 12.5% had 3 resistance genes (Table 3). Only one isolate was negative for all ESC-R genes that were tested. Six farms were *bla*_{CTXM} positive; of the phylogenetic groups 2 farms were *bla*_{CTXM-1} positive, 4 farms were *bla*_{CTXM-9} positive, and 3 farms were *bla*_{CTXM-2} positive. Both *bla*_{CMY-2} and *bla*_{TEM} resistance genes were detected on all 8 farms. In the 12 sESC-R,

E. coli isolates that were ESC susceptible on MIC testing, 11 (11) isolates were *bla*_{TEM} positive, and 4 of these 11 isolates were also *bla*_{CMY-2} positive.

PMQR Genes in ESC-R *E. coli* with Quinolone Resistance Based on MIC Testing

Based on the MIC testing, 9 isolates were nalidixic acid resistant and also had an intermediate susceptibility to ciprofloxacin. Plasmid-mediated quinolone resistance genes were detected from 7 of the 9 isolates. Only 5 isolates were positive for *qnrB* alone; 2 isolates were positive for both *qnrB* and *qnrS*, whereas 2 other isolates were negative for any PMQR genes.

Table 3. β -Lactamase resistance genes patterns in extended-spectrum cephalosporin-resistant *Escherichia coli* isolated from Holstein dairy calves (n = 88) from 8 herds in New Brunswick, Canada (2014–2015)

β -Lactamase pattern	Frequency	%
<i>bla</i> _{CMY-2} <i>bla</i> _{TEM}	25	28.41
<i>bla</i> _{TEM}	21	23.86
<i>bla</i> _{CMY-2}	13	14.77
<i>bla</i> _{TEM} <i>bla</i> _{CTXM-9}	10	11.36
<i>bla</i> _{CMY-2} <i>bla</i> _{TEM} <i>bla</i> _{CTXM-9}	4	4.55
<i>bla</i> _{TEM} <i>bla</i> _{CTXM-1}	4	4.55
<i>bla</i> _{TEM} <i>bla</i> _{CTXM-1} <i>bla</i> _{CTXM-2}	3	3.41
<i>bla</i> _{TEM} <i>bla</i> _{CTXM-2}	3	3.41
<i>bla</i> _{CMY-2} <i>bla</i> _{TEM} <i>bla</i> _{CTXM-2}	2	2.27
<i>bla</i> _{CMY-2} <i>bla</i> _{TEM} <i>bla</i> _{SHV}	1	1.14
<i>bla</i> _{CMY-2} <i>bla</i> _{TEM} <i>bla</i> _{CTXM-1}	1	1.14
β -Lactamase negative	1	1.14

Risk Factors for the Fecal Recovery of *Salmonella enterica* and sESC-R *E. coli*

Descriptive Analysis. The herd demographic information and characteristics of the 8 farms in this study are presented in Tables 4 and 5. The number of cows in all the 8 herds ranged from 76 to 700 lactating cows with an average of 236 cows per herd, whereas the number of neonatal calves ranged from 6 to 45 with an average of 23 per herd. Most of the herds were freestall farms (6/8), and 5 herds housed neonatal calves in hutches. Five of the 8 herds housed sick cattle separately from others. In 5 herds, calves were fed unpasteurized waste milk. None of the herds had any previ-

Table 4. Herd and calf level continuous variable descriptions of the 8 dairy farms from New Brunswick, Canada (2014–2015)

Variable	Average (range)
No. of cows/herd	236 (76–700)
No. of preweaned calves/herd	23 (6–45)
Milk rolling (kg)	8,884.4 (1,900–12,000)
Milk/cow per d (kg)	34.2 (29.5–38)
SCC (cfu/mL)	162,500 (70,000–300,000)

ous history of salmonellosis on the farms. Ceftiofur was used in all herds and was used for the management of diseases such as lameness (2 herds), respiratory diseases (4 herds), reproductive diseases (4 herds), and foot rot (3 herds). Ceftiofur was regularly used in 6 of the herds (Ceftiofur use equal to daily, weekly, or monthly use was considered regular use, whereas occasional use was considered as use at a point in time that was greater than 1 mo). Other antimicrobials reportedly used for the treatments of diseases on the farms were florfenicol (4 herds), penicillin (2 herds), tulathromycin (1 herd), and trimethoprim-sulfadoxine (1 herd).

Risk Factors for the Fecal Recovery of *Salmonella enterica*. Calf age, feeding of unpasteurized waste milk, separation of sick cows from other cattle, and use of ceftiofur for the treatment of respiratory diseases were significantly associated with the fecal re-

covery of *Salmonella enterica* ($P \leq 0.05$) in the univariable regression model. However, other factors including use of penicillin, use of florfenicol, use of ceftiofur for the treatment of scours, and use of ceftiofur for the treatment of lameness were associated with the fecal recovery of *Salmonella enterica* at $P \leq 0.20$ in the univariable analysis (Table 6). Due to the low number of isolates, a multivariable model could not be fitted for this outcome.

Risk Factors for the Fecal Recovery of *sESC-R E. coli*. For *sESC-R E. coli* in calves' feces, average number of cows per herd, average number of calves per herd, lactating cow housing, calf age at sampling, feeding of unpasteurized nonsalable milk, frequency of ceftiofur use, disease conditions for the use of ceftiofur on the farms (lameness, scours, respiratory diseases, foot rot, metritis and mastitis), and the use of other antimicrobials (florfenicol, penicillin, tulathromycin, and trimethoprim-sulfadoxine) were unconditionally associated ($P \leq 0.25$) with fecal recovery of *sESC-R E. coli* at the univariable level (Table 7).

A high correlation was observed between the feeding of unpasteurized nonsalable milk, lactating cow housing, and disease conditions for the use of ceftiofur on the farms. Therefore, feeding of unpasteurized nonsalable milk was used in the subsequent model

Table 5. Herd and calf level categorical variable descriptions of the 8 dairy farms from New Brunswick, Canada (2014–2015)

Variable	Category	Frequency (%)
Breed of cattle on the farms	Holstein	8 (100.0)
Preweaned calves' housing	Calf hutch	5 (62.5)
	Group	3 (37.5)
Lactating cow housing	Freestall	6 (75.0)
	Tiestall	2 (25.0)
Sick cows housed separately from other cows	Yes	5 (62.5)
	No	3 (37.5)
Feeding of unpasteurized milk to calves	Yes	5 (62.5)
	No	3 (37.5)
Previous history of salmonellosis on farms	Yes	0 (0.0)
	No	8 (100.0)
Ceftiofur use on the farms	Yes	8 (100)
	No	0 (0.0)
Frequency of ceftiofur use on the farms	Regularly	6 (75.0)
	Occasionally	2 (25.0)
Previous history of ceftiofur use on the farm for treatment of salmonellosis	Yes	0 (0.0)
	No	8 (100.0)
Use of ceftiofur for the management of other diseases on the farms	Yes	8 (100.0)
	No	0 (0.0)
Disease conditions in which ceftiofur was used on the farms	Lameness	2 (25.0)
	Respiratory diseases	4 (50.0)
	Scours	2 (25.0)
	Metritis/retained placenta	5 (62.5)
	Anorexia/general infection	2 (25.0)
	Footrot	3 (37.5)
	Mastitis	1 (12.5)
Other antimicrobials used for the treatments of diseases on the farm within the last 6 mo	Florfenicol	4 (50.0)
	Penicillin	2 (25.0)
	Trimethoprim-sulfadoxine	1 (12.5)
	Tulathromycin	1 (12.5)

building process because previous reports documented the relationship between the feeding of unpasteurized nonsalable milk and fecal shedding MDR bacteria in dairy calves. Additionally, collinearity was also observed between penicillin, florfenicol, tulathromycin, and trimethoprim-sulfadoxine. Florfenicol was selected for subsequent analysis. Four covariates from 4 farms were influential in the model building. Three of these were from the farms that used ceftiofur regularly as well as fed unpasteurized nonsalable milk. The fourth observation was from a farm that also used ceftiofur regularly but did not feed unpasteurized nonsalable milk to calves. One of these covariates produced a lack of fit and had a strong effect on the coefficient of use of ceftiofur for the treatment of respiratory diseases. This influential covariate was from a herd that fed waste milk to calves and used ceftiofur regularly. However, it was negative for sESC-R *E. coli* and was from fecal samples of calves during the weaning period. Based on predictability and reliability of the model, the model was highly sensitive (86.52%), but less specific (40%).

Five variables remained in the multivariable model and were significantly associated with recovery of sESC-R *E. coli* from calf feces (Table 8). None of the

interaction terms among the variables were significant. Recovery of sESC-R *E. coli* was more likely in neonates than those of weaning age [odds ratio (OR) = 2.42, 95% CI: 1.87–3.12], farms reporting regular ceftiofur use versus occasional use (OR = 3.83, 95% CI: 2.29–6.39), farms feeding unpasteurized nonsalable milk to calves (OR = 1.61, 95% CI: 1.18–2.18), and the use of florfenicol (OR = 2.02, 95% CI: 1.43–2.86). Recovery of sESC-R *E. coli* was less likely when ceftiofur was used versus nonuse for the treatment of respiratory diseases (OR = 0.57, 95% CI: 0.41–0.79).

DISCUSSION

The frequency of fecal recovery of *Salmonella enterica* in this study was low, although similar to the low prevalence reported in Alberta, Canada (Sorensen et al., 2003), but lower than that reported by Abouzeed et al. (2000) in Prince Edward Island beef cattle. This low frequency in addition to the pansusceptibility of recovered isolates to tested antimicrobials may suggest that *Salmonella enterica* is of low risk to dairy cattle within this region. Host-specific *Salmonella* serovars Dublin and other common serovars commonly isolated from

Table 6. Univariable logistic regression of variables unconditionally associated with fecal recovery of *Salmonella enterica* (2014–2015)¹

Variable	Category	Frequency (%)	Odds ratio	Robust SE	P-value
No. of calves/herd	Small	54.7	— ²	—	—
	Larger	45.3	2.53	1.44	0.105
Age of preweaned calf at sampling (d)	45–56	50.0	—	—	—
	2–15	50.0	4.10	1.94	0.003
Preweaned calves' housing	Calf hutch	63.7	—	—	—
	Group	36.3	0.75	0.41	0.601
Lactating cow housing	Freestall	67.6	0.89	0.50	0.85
	Tiestall	32.4	1.29	2.768	0.902
Sick cows housed separately from other cows	No	28.1	—	—	—
	Yes	71.9	0.25	0.13	0.008
Feeding of unpasteurized milk to calves	No	38.9	—	—	—
	Yes	61.1	0.27	0.16	0.025
Frequency of ceftiofur use	Occasionally	11.9	—	—	—
	Regularly	88.1	0.76	0.54	0.701
Use of ceftiofur for lameness	No	66.0	—	—	—
	Yes	34.0	2.42	1.31	0.104
Use of ceftiofur for respiratory diseases	No	62.9	—	—	—
	Yes	37.1	5.30	2.92	0.003
Use of ceftiofur for scours	No	75.6	—	—	—
	Yes	24.4	2.10	1.11	0.158
Use of ceftiofur for metritis/retained placenta	No	59.8	—	—	—
	Yes	40.2	1.22	0.62	0.691
Use of ceftiofur for anorexia/general infection	No	73.9	—	—	—
	Yes	26.1	3.58	1.82	0.012
Use of ceftiofur for foot rot	No	73.4	—	—	—
	Yes	26.6	0.48	0.35	0.314
Florfenicol	No	61.3	—	—	—
	Yes	38.7	2.42	1.26	0.089
Penicillin	No	82.8	—	—	—
	Yes	17.2	2.1	1.15	0.175

¹Observations for number of cows/herd, trimethoprim-sulfadoxine, tulathromycin, and mastitis were too low to allow for analysis. Number of calves/herd: small: <23 calves/herds, larger: ≥23 calves/herd.

²—: reference.

Table 7. Univariable logistic regression of variables unconditionally associated with fecal recovery of suspected extended-spectrum cephalosporin-resistant *Escherichia coli* (2014–2015)¹

Variable	Category	Frequency (%)	Odds ratio	Robust SE	P-value
No. of calves/herd	Small	54.7	— ²	—	—
	Larger	45.3	0.77	0.11	0.07
No. of cows/herd	Small	74.2	—	—	—
	Larger	25.8	2.0	0.34	<0.001
Age of preweaned calf at sampling (d)	45–56	50.0	—	—	—
	2–15	50.0	2.27	0.27	<0.001
Preweaned calves' housing	Calf hutch	63.7	—	—	—
	Group	36.3	1.10	0.16	0.526
Lactating cow housing	Freestall	67.6	—	—	—
	Tiestall	32.4	2.1	0.33	<0.001
Sick cows housed separately from other cows	No	28.1	—	—	—
	Yes	71.9	0.89	0.14	0.475
Feeding of unpasteurized milk to calves	No	38.9	—	—	—
	Yes	61.1	1.72	0.25	<0.001
Frequency of ceftiofur use	Occasionally	11.9	—	—	—
	Regularly	88.1	2.89	0.62	<0.001
Use of ceftiofur for lameness	No	66.0	—	—	—
	Yes	34.0	0.83	0.12	0.226
Use of ceftiofur for respiratory diseases	No	62.9	—	—	—
	Yes	37.1	0.66	0.10	0.004
Use of ceftiofur for scours	No	75.6	—	—	—
	Yes	24.4	0.45	0.07	<0.001
Use of ceftiofur for metritis/retained placenta	No	59.8	—	—	—
	Yes	40.2	0.71	0.10	0.016
Use of ceftiofur for anorexia/general infection	No	73.9	—	—	—
	Yes	26.1	0.85	0.13	0.305
Use of ceftiofur for foot rot	No	73.4	—	—	—
	Yes	26.6	0.65	0.10	0.006
Use of ceftiofur for mastitis	No	74.2	—	—	—
	Yes	25.8	2.0	0.34	<0.001
Florfenicol	No	61.3	—	—	—
	Yes	38.7	1.43	0.21	0.016
Penicillin	No	82.8	—	—	—
	Yes	17.2	1.96	0.40	0.001
Trimethoprim-sulfadoxine	No	89.3	—	—	—
	Yes	10.7	2.0	0.51	0.007
Tulathromycin	No	88.9	—	—	—
	Yes	11.1	0.56	0.12	0.009

¹Number of calves/herd: small, <23 calves/herds; larger, ≥23 calves/herd.²—: reference.

both healthy and diarrheic cattle such as Newport, Typhimurium DT 104, and Montevideo in North America (Abouzeed et al., 2000; Wells et al., 2001; Berge et al., 2006a) were not isolated in this study. All of the isolates

serotyped belonged to 3 serovars: Senftenberg, Typhimurium DT02, and Derby. To our knowledge, this is the first reported isolation of *Salmonella* Typhimurium DT02 in dairy cattle in Canada. Although *Salmonella*

Table 8. Population average multivariable logistic regression of predictors associated with fecal recovery of suspected extended-spectrum cephalosporin-resistant *Escherichia coli* (2014–2015)

Variable	Level	Odds ratio	Robust SE	P-value
Age of preweaned calf at sampling (d)	45–56	— ¹	—	—
	2–15	2.42	0.31	<0.001
Frequency of ceftiofur use	Occasionally	—	—	—
	Regularly	3.83	1.00	<0.001
Feeding of unpasteurized nonsalable milk	No	—	—	—
	Yes	1.61	0.25	0.002
Use of florfenicol	No	—	—	—
	Yes	2.02	0.36	<0.001
Use of ceftiofur for the treatment of respiratory diseases	No	—	—	—
	Yes	0.57	0.09	0.001

¹—: reference.

Senftenberg has been isolated from dairy cattle, this serovar is considered to be less important and one of the infrequent serotypes of nonclinical bovine isolates (Centers for Disease Control and Prevention, 2002). However, it was reported recently as one of the serovars of nontyphoidal salmonellosis in humans (Centers for Disease Control and Prevention, 2016).

Salmonella Derby isolated from dairy calves in this study is more host specific and adapted to pigs and is reported to cause clinical salmonella infections in humans (Philippeczik et al., 1999). *Salmonella* Typhimurium DT02 has a narrow host range and more commonly causes infections in pigeons (Rabsch, 2002). The isolation of *Salmonella* Typhimurium DT02 in dairy calves may further support the importance of birds that frequent dairy farms in transmitting disease-causing pathogens as well as being reservoirs of foodborne pathogens (Kirk et al., 2002). The *Salmonella enterica* isolates recovered in this study were pansusceptible, which is consistent with other published reports both in Canada (Sorensen et al., 2003) and the United States (Berge et al., 2006a).

Different studies have reported on the prevalence of sESC-R *E. coli* in dairy calves using selective culture. Eighty-one percent (81%) of calves were positive for sESC-R *E. coli* in this study, which was lower than the 93 and 88% reported by Donaldson (Donaldson et al., 2006) and Davis (Davis et al., 2015), respectively, in the United States. Fecal prevalences of 34.4 to 61% have been reported from Ohio dairy cattle (Tragesser et al., 2006; Heider et al., 2009a). Previous studies have reported that calves are colonized with MDR bacteria including *Escherichia coli* at an early age with subsequent shedding, especially within the first 2 wk of life (Berge et al., 2005; Donaldson et al., 2006; Edrington et al., 2012).

In this study, 88% of the selected sESC-R-*E. coli* isolates that grew on VACC agar demonstrated phenotypic resistance to ESC (ceftriaxone, ceftiofur, or both) on MIC testing. These ESC-R isolates also had co-resistance to at least 3 or more other antimicrobial categories, thus fitting the definition of MDR isolates. The most common co-resistance with ESC was with streptomycin, sulfisoxazole, trimethoprim-sulfamethoxazole, and tetracycline versus the commonly reported co-resistance to fluoroquinolones and aminoglycosides (Cantón et al., 2012; Seiffert et al., 2013).

Four different β -lactamase genes were detected in this study belonging to Ambler molecular classes A (*bla*_{TEM}, *bla*_{CTX-M}, and *bla*_{SHV}) and C (*bla*_{CMY-2}). These β -lactamase resistance genes have also been recently reported from beef cattle in Alberta, Canada, however, at varying proportions compared with this study (Cormier et al., 2015). Detection of *bla*_{TEM}, *bla*_{CMY-2}, *bla*_{CTX-M}, and

*bla*_{SHV} explains most of the causes of the ESC-R in *E. coli* isolates in this study. Nondetection of these ESC resistance genes in one ESC-R *E. coli* isolate suggests the presence of other mechanisms of ESC resistance, such as efflux pumps or chromosomal-mediated resistance determinants. The 12 sESC-R *E. coli* isolates that did not have phenotypic evidence of ESC resistance based on MIC testing were resistant to ampicillin, amoxicillin-clavulanate (β -lactam/ β -lactamase inhibitor combination), and cefoxitin. This β -lactam/ β -lactamase inhibitor resistance may indicate that the *bla*_{TEM} resistance genes detected in the ESC-susceptible isolates were not ESBL but may be inhibitor-resistant *bla*_{TEM}. However, the presence of cefoxitin resistance in all the suspected inhibitor resistant *bla*_{TEM} isolates and detection of the *bla*_{CMY-2} resistance gene in another 4 isolates may suggest the existence of 2 or more β -lactamase resistance mechanisms (Cantón et al., 2008).

The ESC-R in cattle within North America has been attributed mainly to the *bla*_{CMY-2} resistance gene (Donaldson et al., 2006; Mollenkopf et al., 2012; Cormier et al., 2015). Even though *bla*_{CMY-2} was detected from all the farms, *bla*_{TEM} was the more commonly observed β -lactamase resistance gene in this study.

Previous studies within Canada have characterized and reported the *bla*_{CMY-2} gene and its associated plasmid dynamics in *S. enterica* and *E. coli* from human, cattle, environmental sources, and meat products (Forward et al., 2004; Mulvey et al., 2009; Mataseje et al., 2010; Martin et al., 2012). However, compared with *bla*_{CMY-2}, studies on *bla*_{CTX-M} resistance genes in Canadian cattle are limited. In Europe, the *bla*_{CTX-M} resistance gene is known and reported to be the most common cause of ESC resistance in the *Enterobacteriaceae* (Seiffert et al., 2013). No *bla*_{CTX-M} resistance genes were reported from animals in Canada over a decade ago (Li et al., 2007), although they were commonly reported in *E. coli* as a major cause of community-acquired infections in humans (Pitout et al., 2004b). In this study, 30.7% of selected sESC-R *E. coli* isolates were carriers of *bla*_{CTX-M} resistance genes. To the best of our knowledge, this is the first report of detection of *bla*_{CTX-M} resistance genes in dairy calves within Atlantic Canada.

Two different groups of *bla*_{CTX-M} were detected in this study (groups 1 and 9). They are the most widely distributed *bla*_{CTX-M} groups in both human and animal bacterial isolates, especially *bla*_{CTX-M-15} of group 1 and *bla*_{CTX-M-14} of group 9 in North America and other parts of the world (Pitout et al., 2004a; Cantón et al., 2012). The detection of *bla*_{CTX-M} in this study together with other recent reports from beef cattle from western Canada (Cormier et al., 2015) and dairy cattle from the United States (Wittum et al., 2010; Mollenkopf et al., 2012; Davis et al., 2015) may suggest the pos-

sible emergence of *bla*_{CTX-M}-producing *Escherichia coli* within North America. The coexistence of 2 or more β -lactamases was detected in 44% of the MIC-confirmed ESC-R *E. coli*. This may explain how more than one resistance gene plays a critical role in the appearance of phenotypic resistance characteristics.

The PMQR genes were detected in 7 of 9 quinolone-resistant isolates, and to the best of our knowledge, this is the first detection of PMQR genes in dairy cattle in Canada. These *qnr* genes did not produce increased resistance to fluoroquinolones beyond the MIC breakpoints; however, in the presence of chromosomal mutations in DNA topoisomerase II, several-fold increases in MIC have been reported (Robicsek et al., 2006). The PMQR *B* and *S* were detected in 7 β -lactamase producing isolates with 4 isolates harboring 2 or more β -lactamase resistance genes, usually *bla*_{TEM} and *bla*_{CMY-2}. The PMQR genes, especially *qnrB19*, are known to be frequently associated with other antimicrobial resistance genes especially β -lactamases, with colocation on the same plasmid and other mobile genetic elements (Cattoir et al., 2007).

In this study, calf age was associated with the fecal recovery of sESC-R *E. coli*. The increased frequency in neonates compared with calves at weaning agrees with other reports that prevalence is inversely related to age, with peaked prevalence commonly seen in the first 2 wk of life (Hoyle et al., 2004; Khachatryan et al., 2004; Donaldson et al., 2006; Edrington et al., 2012). A similar finding was observed in *Salmonella enterica*. This change in prevalence is attributed to age-associated changes in the commensal enteric microflora, which results in an intestinal microbiota that is more effective at excluding these bacteria (e.g., sESC-R *E. coli* and *Salmonella enterica*) from the intestinal microcosm (Berge et al., 2006a).

Use of ceftiofur for the treatment of respiratory diseases was protective against shedding of sESC-R *E. coli*; this may suggest the effect of ceftiofur use among the 8 herds may be due to the treatment of other nonrespiratory diseases such as reproductive diseases. Also, there is a possibility of use of other antimicrobials by the farms that were not using ceftiofur, which may be causing resistance selection. However, in the model without the influential covariate; the effect of this predictor was nonsignificant. Florfenicol use was associated with the fecal shedding of sESC-R *E. coli*; although both florfenicol (extra-label use) and ceftiofur (label use) are used for the treatment of respiratory disease in dairy cattle, the reason for the relationship is not clear. Interestingly, the association between the phenotypic florfenicol and third-generation cephalosporin resistance and the co-presence of *bla*_{CMY-2} and *flo-R*

resistance genes on the same plasmid have been previously reported (Meunier et al., 2010).

All the farms sampled reportedly used ceftiofur in some fashion. However, calves from farms with regular ceftiofur use (monthly or more often) were more likely to shed sESC-R *E. coli*, suggesting that the use of this third-generation cephalosporin may drive resistance. Previous studies in different animal species have found that frequent use of antimicrobials is related to increased shedding of MDR bacteria (van den Bogaard and Stobberingh, 2000; Call et al., 2008) including dairy calves (Berge et al., 2006b). Although MDR *E. coli* has been reported in preweaned calves regardless of antimicrobial exposure (Khachatryan et al., 2004), the contribution of other factors apart from antimicrobial use such as unpasteurized nonsalable milk feeding has been reported (Pereira et al., 2014b).

In this study, unpasteurized nonsalable milk feeding was associated with the fecal recovery of sESC-R *E. coli*. Nonsalable milk feeding is a common practice in Canada (Vasseur et al., 2010). This practice has also been reported from other parts of the world including the United States (Pereira et al., 2014b), Sweden (Duse et al., 2013), and the United Kingdom (Brunton et al., 2014). Consistent with this study, the association between unpasteurized nonsalable milk feeding and increased shedding of MDR bacteria has also been reported (Berge et al., 2006a; Aust et al., 2013; Duse et al., 2015). Increased prevalence of MDR *E. coli* was reported to coincide with the period when calves are fed waste milk, suggesting that the fecal recovery in dairy calves may be diet related (Khachatryan et al., 2004). This feeding practice facilitates the exposure of dairy calves to antimicrobials via consumption, thus leading to possible antimicrobial selective pressure and resistance development (Randall et al., 2014).

The low discriminating power of the model used in predicting the association between the variables examined and fecal recovery of sESC-R *E. coli* is a major limitation in this study; with the model specificity of 40%, there is a high probability of misclassification of dairy calves as shedders of ECS-R *E. coli* when in reality they are not. This is unsurprising considering the low number of dairy herds participating in this study. The association between the risk factors that were associated with fecal recovery of sESC-R *E. coli* will need to be further elucidated, and we believe this study can be a framework for a future, more robust, study.

This study has provided estimates of the distribution of ESC-R in dairy calves in New Brunswick, Canada. Using molecular epidemiologic techniques, it has provided information on the genes associated with the occurrence of resistance to ESC in dairy calves within

this region. This study revealed a low frequency of *Salmonella enterica* in dairy calves. A high frequency of sESC-R *E. coli* could be found in calves when using a selective culture method for ESC enteric bacteria. The *Bla*_{CMY-2} and *bla*_{TEM} were the prevalent β -lactamases found in ESC-R *E. coli* isolated from dairy calves. Calves' age, regular ceftiofur use on the farm, feeding of unpasteurized nonsalable milk, use of ceftiofur for treatment of respiratory diseases, and use of florfenicol were factors associated with fecal recovery of *E. coli* with reduced susceptibility to ESC. However, to our knowledge, this is the first report of the occurrence of *qnrB* and *qnrS* genes in *E. coli* isolated from dairy calves in Canada. We believe this will be relevant to the dairy farmers and veterinarians within this region for management purposes and epidemiologic understanding of factors influencing antimicrobial resistance.

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