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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 ($bla_{\text{CTX-M}}$, $bla_{\text{CMY-2}}$, 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%, $bla_{\text{CMY-2}}$ 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 $bla_{\text{CMY-2}}$ and to a lesser extent to the ESBL resistance genes such as $bla_{\text{CTX-M,}}$ 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 $bla_{\text{CTXM-15}}$, $bla_{\text{SHV-5}}$, and $bla_{\text{SHV-7}}$, 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