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Diversity of CTX-M-positive *Escherichia coli* recovered from animals in Canada

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ABSTRACT

Historically, extended-spectrum cephalosporin resistance in bacteria from animals in Canada has been attributed to the SHV and CMY β-lactamase families. This pattern is beginning to change with the emergence of the *bla*$_{\text{CTX-M}}$ gene family among *Escherichia coli* recovered from various animal species. Here we analyze and compare whole genome sequences of *bla*$_{\text{CTX-M}}$-positive *E. coli* isolates (n = 173) from dogs, chicken, swine, horses and beef cattle in Canada. Ten *bla*$_{\text{CTX-M}}$ variants were identified with *bla*$_{\text{CTX-M-1,-14, -15, -27}}$ and *bla*$_{\text{CTX-M-55}}$ being identified in most animal species. These variants occurred across many sequence types, suggesting that mobile genetic elements mediate the spread of *bla*$_{\text{CTX-M}}$. The variants *bla*$_{\text{CTX-M-14, -15, -27}}$ and *bla*$_{\text{CTX-M-55}}$ are associated with the global spread of *bla*$_{\text{CTX-M}}$ in human clinical isolates and their presence could be indicative of transfer between humans and animals. These variants were also the principal variants identified among sequence type 131 isolates, which were not associated with any other species than dogs. These isolates carried the same *bla*$_{\text{CTX-M}}$ variants as *E. coli* isolates found in humans. Close contact may promote the transmission of these isolates between humans and companion animals.

1. Introduction

Commensal *Escherichia coli* have been suggested as a potential reservoir for extended-spectrum cephalosporin (ESC) resistance (Lalak et al., 2016). Due to the ability of these bacteria to colonize the digestive tracts of both humans and animals, their transmission through contaminated food or direct contact is a public health concern. ESC resistance in *E. coli* is most commonly caused by the production of β-lactamases encoded by the *bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV}}$, and *bla*$_{\text{CMY}}$ gene families. These genes are frequently found on mobile genetic elements, such as plasmids and transposons, aiding in their transmission and potential transfers to pathogenic strains (Lalak et al., 2016).

Humans and dogs in Canada first saw the emergence of *bla*$_{\text{CTX-M}}$ in the early 2000's (Boyd et al., 2004; Mulvey et al., 2004; Kashayar, 2009). ESC resistance in bacteria from Canadian farm animals has been attributed to the SHV and CMY β-lactamase families (Martin et al., 2012; Pouget et al., 2013). More recently, *bla*$_{\text{CTX-M}}$ variants have been observed in bacterial isolates recovered from a wider range of animal species, such as poultry, beef cattle and swine (Cormier et al., 2016; Chalmers et al., 2017; Zhang et al., 2018). Knowledge of strain diversity and *bla*$_{\text{CTX-M}}$ variants among these isolates could shed light on the spread of ESC resistance in Canada. The objectives of this study were to use whole genome sequences to obtain and compile preliminary information on the current diversity of *bla*$_{\text{CTX-M}}$-carrying *E. coli* and their respective gene variants in isolates from chicken, swine, beef cattle, horses, and dogs from Canada.

2. Materials and methods

One hundred and seventy-three *bla*$_{\text{CTX-M}}$-positive *E. coli* isolates were used in this analysis. The sequences from 42 isolates were from dogs (Zhang et al., 2018), 22 from swine (Zhang, 2017), 66 from beef cattle (manuscript under review), 32 from chickens and 11 from horses. All were from fecal, cecal, or diagnostic samples collected between 2014 and 2017. Dog, chicken, swine and horse samples were collected in Ontario, and beef cattle samples were collected in Alberta. Isolates recovered from chicken and swine originated from cecal samples, whereas isolates from horses and beef cattle originated from fecal

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samples. Isolates from dogs originated from fecal and a variety of diagnostic samples. Samples from dogs, swine, chicken and cattle were cultured using enrichment broth containing cefotaxime (2 μg/mL) and isolates were selected using subcultures on agar supplemented with ceftriaxone (1 μg/mL). Diagnostic isolates from dogs were collected without the use of enrichment (Zhang et al., 2018). Fecal samples from horses were collected at a single major racetrack over two different days. Isolates were incubated overnight in LB broth without antibiotics and isolates selected using CHROMID® ESBL agar (BioMérieux, Laval, QC, Canada).

DNA extractions were performed using the Epicentre MasterPure DNA Purification kit (Epicentre, Madison, WI, USA). Sequences were obtained using MiSeq (PE300) and NextSeq technology (Illumina, San Diego, CA, USA; Advanced Analysis Centre) following library preparation using Nextera XT kits (Illumina). Whole genome sequences were assembled using the BioNumerics v7.6 (Applied Maths, Austin, TX, USA), SPAdes algorithm with assembly-free and assembly-based allele calling. Sequence types (STs) were assigned using the whole genome multi-locus sequence typing (wgMLST) plug-in and the *E. coli/Shigella* Enterobase scheme. The CTX-M genes,their respective variants, and virulence genes were identified using the *E. coli* functional genotyping application in BioNumerics v7.6. Minimum spanning trees (MSTs) were created using core genome multi-locus sequencing typing (cgMLST) to observe the relatedness of the isolates.

3. Results

Ten bla_{CTX-M} variants were identified from 173 isolates (Table 1). Isolates from dogs presented the greatest variety of bla_{CTX-M} variants (n = 8), followed by feedlot cattle (n = 7), swine (n = 5), chickens (n = 4), and horses (n = 1). The $bla_{CTX-M-1}$ variant was the dominant variant found in chicken (91%) and horse (100%) isolates. The remaining bla_{CTX-M} variants appear to be more heterogeneous among *E. coli* isolated from cattle, swine and dogs.

Minimum spanning trees were created using cgMLST and 1998 loci. Based on the distribution of branch lengths, clusters were defined as a group of three or more isolates connected by a branch length of less than 100 locus differences. Some clustering was observed among isolates from the same animal species (Fig.1). In contrast, most isolates within a species were largely unrelated to those collected from other animal species. Nevertheless, four canine isolates were less than 20 locus variations apart from isolates collected from swine, chicken, and cattle (Fig. 1). They also appeared to share the same bla_{CTX-M} variant ($bla_{CTX-M-1,-15}$ and $bla_{CTX-M-55}$) (Fig. 2). Sixty-two unique STs were identified among the 173 isolates using the Achtman sequence typing scheme (Table S1). The most prevalent STs in this study (ST224, and ST683) and the STs known to have the most clinical relevance (ST10, ST117, ST131 and ST648) are depicted in Fig. 3. Five of the six major STs were associated with a specific animal species ST117 (chicken), ST131 (dog), ST224 (beef cattle), ST648 (dog), and ST683 (beef cattle) (Figs. 1 and 3). More than one bla_{CTX-M} variant was observed in the ST10, ST131, and ST648 clusters. Sequence type 131, arguably the most clinically relevant in humans, was not found in any other species than dogs. STs 224 and 683 were only identified among beef cattle isolates with either $bla_{CTX-M-55}$ or $bla_{CTX-M-65}$, respectively, with the exception of a single canine isolate that was also part of the $bla_{CTX-M-55}$ cluster. Isolates found to be ST 117, which has been commonly found among avian pathogenic *E. coli* (APEC), carried many virulence genes typically associated with this pathotype (including *iss*, *iroN*, *OmpT*, *ireA*, *fyuA*, *iutA*, *tsh*, *papC*, and *kpsII*). Most other variants ($bla_{CTX-M-1,\ -14,\ -15,}$ and $bla_{CTX-M-27}$) were found across a wide range of sequence types and animal sources (Fig. 1, Fig. 2 and Table S1).

4. Discussion

The majority of bla_{CTX-M} variants identified in this analysis were found in *E. coli* from more than one animal species, and have been identified in humans and animals globally (Bevan et al., 2017). The presence of the same variants across bacteria from several animal species and in multiple STs suggested that mobile genetic elements play an important role in the spread of bla_{CTX-M}. It may also allude to multiple and repeated entry of bla_{CTX-M}-carrying strains into animal populations in Canada since their first appearance in the early 2000’s. Most noteworthy of these are the $bla_{CTX-M-14,-15,\ -27,}$ and $bla_{CTX-M-55}$ variants, which have been associated with the global spread of bla_{CTX-M} in humans (Bevan et al., 2017). Although found in isolates from every animal species investigated, $bla_{CTX-M-1}$ was overwhelmingly dominant in *E. coli* from chickens and horses. This trend has also been observed in Europe (Girlich et al., 2007; Dierikx et al., 2012). It may suggest that the genetic elements carrying this variant result in a particular selective advantage in these animal species, either through colonization, metabolic adaptation to the host digestive tract or increased environmental stability.

The number of bla_{CTX-M} variants and STs observed among each animal species may be in part reflective of the environment in which these animals are raised. For example, most dogs live in a complex environment where exposure to a variety of people, animals and foods is common, possibly increasing the acquisition of various bacterial strains. This is a likely contributor to the higher diversity of bla_{CTX-M} variants observed with this species. In contrast, production animals live in more confined and standardized environments. For example, swine and poultry are often part of integrated production lines with strict biosecurity control measures and are therefore less likely to see the introduction of organisms and strains from a broad variety of sources in their environment. The lack of bla_{CTX-M} variant diversity seen among horse isolates may be a result of fecal samples being collected from a single location. However, the extensive strain diversity recovered from these animals would suggest either an extremely active horizontal transfer of $bla_{CTX-M-1}$ within strains at this location or acquisition from multiple sources. Although a larger collection of isolates from various regions is necessary to accurately assess the diversity of bla_{CTX-M} variants among *E. coli* from Canadian horses, our results strongly suggest

Table 1
The number and proportion of each CTX-M variant identified among *Escherichia coli* isolates (n = 173), from chicken (n = 32), swine (n = 22), beef cattle (n = 66), dogs (n = 42), and horses (n = 11).

CTX-M variant	Chicken	Swine	Beef cattle	Dog	Horse	Total
CTX-M-1	29 (91%)	9 (41%)	3 (5%)	6 (14%)	11 (100%)	58 (34%)
CTX-M-14	1 (3%)	8 (36%)	1 (2%)	5 (12%)	–	15 (9%)
CTX-M-15	1 (3%)	2 (9%)	11 (17%)	12 (29%)	–	26 (15%)
CTX-M-27	–	2 (9%)	2 (3%)	9 (21%)	–	13 (8%)
CTX-M-32	–	–	1 (2%)	–	–	1 (1%)
CTX-M-55	1 (3%)	–	30 (45%)	7 (17%)	–	38 (21%)
CTX-M-65	–	–	18 (27%)	–	–	18 (10%)
CTX-M-115	–	1 (5%)	–	1 (2%)	–	2 (1%)
CTX-M-169	–	–	–	1 (2%)	–	1 (1%)
CTX-M-202	–	–	–	1 (2%)	–	1 (1%)

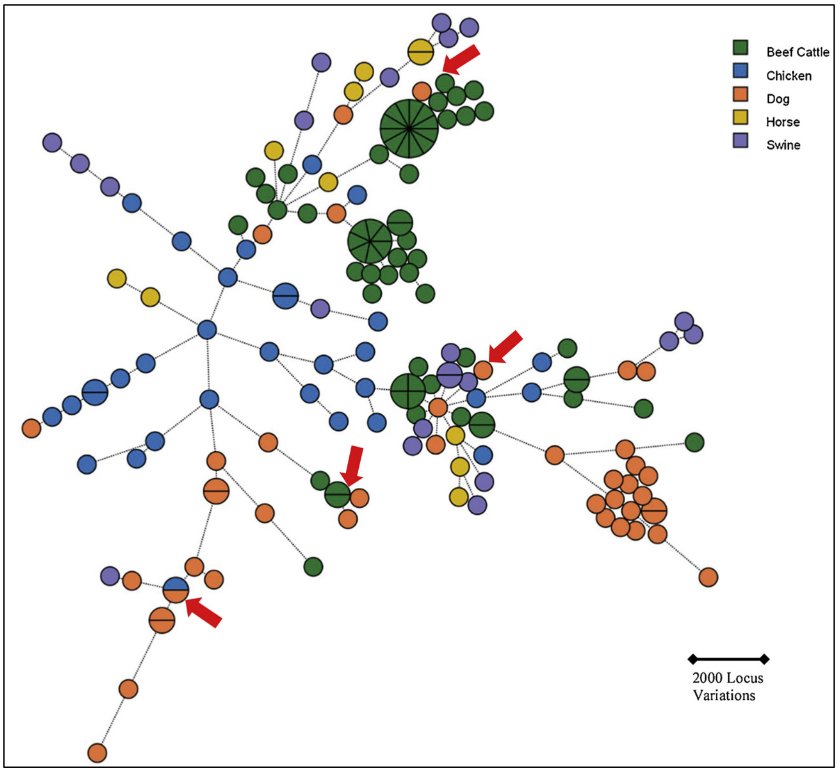

Fig. 1. Minimum spanning tree created using cgMLST, comparing 1998 loci colored by animal species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
*Red arrows indicate the four canine isolates that are less than 20 locus variations apart from production animal isolates
*Clusters were defined as a group of three or more isolates connected by a branch length of less than 100 locus differences

that *bla*$_{CTX-M-1}$ may be the dominant variant among bacterial isolates from this species. This is similar to findings from other groups in Europe (Dierikx et al., 2012; Lupo et al., 2018), and to another study in Nova Scotia, Canada where an isolate carrying *bla*$_{CTX-M-1}$ was the only ESC-resistant isolate identified in horses (Timonin et al., 2016).

Sequence type 10, ST131, and ST648 have been associated with the global spread of *bla*$_{CTX-M}$ in humans and animals (Manges and Johnson, 2012; Pitout, 2012; Nicolas-Chanoine et al., 2014) and may be reflective of their long-standing presence and the role of horizontal gene transfer in the spread of *bla*$_{CTX-M}$. Identifying STs generally associated with humans among canine isolates, and not in other animal species is probably reflective of the less direct interactions between humans and

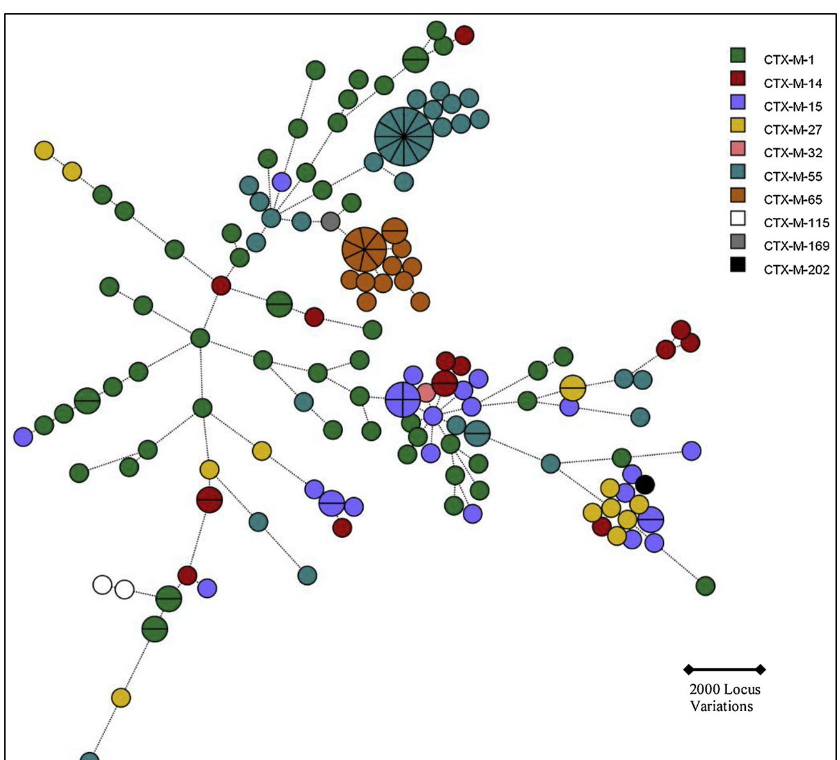

Fig. 2. Minimum spanning tree created using cgMLST, comparing 1998 loci colored by CTX-M variant.

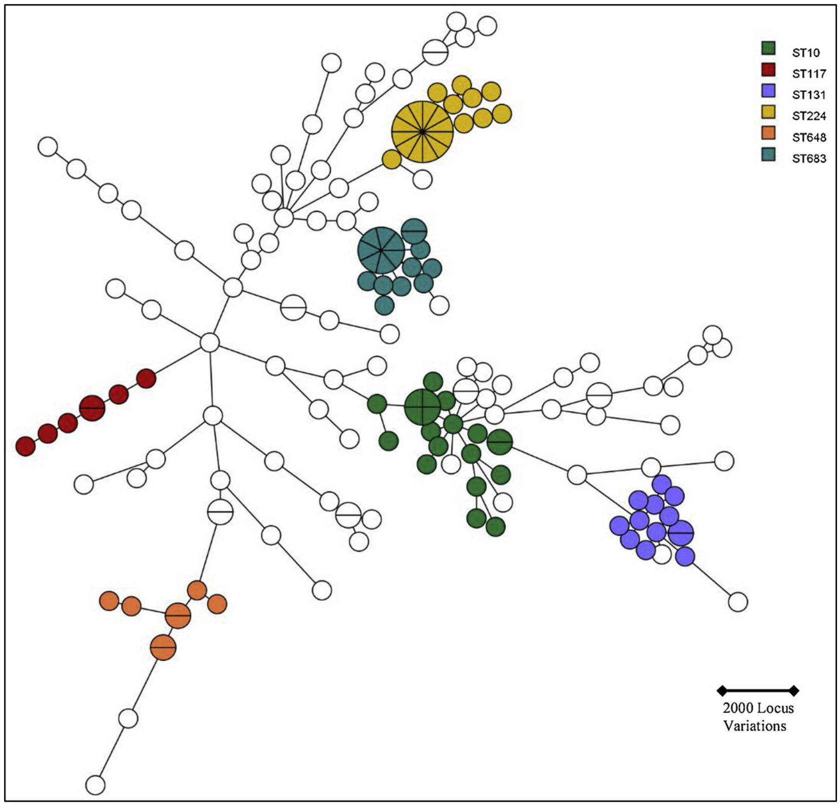

Fig. 3. Minimum spanning tree created using cgMLST, comparing 1998 loci colored by major sequence type of interest.

farm animals, as opposed to the close relationships that humans have with their dogs. Sequence type 224 and ST683 are not as widespread as ST10, ST131, or ST648. However, they do represent the dominant STs found among beef cattle isolates in this study. Isolates from beef cattle were collected from four feedlots that carried out similar production practices and therefore the dominance of these STs is likely reflective of clonal expansion or similar selective pressures within these feedlots (manuscript under review). *Escherichia coli* ST117, which was the most frequent ST identified from chickens, has been associated with clinical infections in humans (Bergeron et al., 2012; Ho et al., 2012). It is also widespread among APEC isolates and has been associated with outbreaks of avian colibacillosis (Mora et al., 2012; Ronco et al., 2017). The ST117 isolates in this study, although of cecal (chicken) or fecal (dog) origin, also appear to carry virulence genes typically associated with APEC. The tendency of this ST to be associated with $bla_{\text{CTX-M-1}}$, and virulence factors involved in the pathogenesis of infections in humans and poultry may be cause for concern, since this may lead to the spread of virulent strains resistant to third and fourth generation cephalosporins. When analyzed using MST, some canine isolates were found to be genetically similar to those from chickens, swine and cattle, whereas isolates across production animals did not cluster. This further supports our hypothesis that the types of environment animals reside in have a significant effect on the variety of organisms that they may come in to contact with during their life.

In conclusion, $bla_{\text{CTX-M}}$-positive isolates from different Canadian animal species do not appear to be closely related. However, the same $bla_{\text{CTX-M}}$ variants are frequently found in more than one animal source. This suggests that these genes are spreading efficiently through bacterial populations to livestock and companion animals or that $bla_{\text{CTX-M}}$-positive bacteria are entering production animal industries from diverse sources. Specific sequence types (ST131, ST117, ST224 and ST683) and some gene variants ($bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-55}}$, and $bla_{\text{CTX-M-65}}$) were more abundant in some animal species than others, and may further indicate that $bla_{\text{CTX-M}}$-carrying isolates are entering these ecological niches from sources unique to each species or industry. Additional isolates from a wider range of locations and sources are necessary to confirm the trends found in this study and fully characterize the current diversity of $bla_{\text{CTX-M}}$-carrying *E. coli* in Canada. Plasmid characterization should also be pursued to further assess how $bla_{\text{CTX-M}}$ genes are spreading through Canadian livestock and companion animals.

Conflict of interests

There are no competing interests to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2019.02.031.

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