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bla$_{CTX-M-32}$ on an IncN Plasmid in *Escherichia coli* from Beef Cattle in the United States

Jennifer L. Cottell,[a] Neena Kanwar,[b] Lucrecia Castillo-Courtade,[a] Gabhan Chalmers,[a] H. Morgan Scott,[b] Bo Norby,[c] Guy H. Loneragan,[d] Patrick Boerlin[a]

Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada[a]; Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, Kansas, USA[b]; Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, Michigan, USA[c]; International Center for Food Industry Excellence, Department of Animal and Food Sciences, Texas Tech University, Lubbock, Texas, USA[d]

CTX-M-type enzymes are the most extensively distributed extended-spectrum β-lactamases (ESBLs) conferring resistance to advanced-generation cephalosporins in *Enterobacteriaceae* worldwide (1). While these enzymes have also become the most common ESBLs detected in U.S. human infections (2, 3), data on *bla*$_{CTX-M}$ genes in bacteria from U.S. livestock still remain scarce (4, 5).

In this report, we describe the detection of *bla*$_{CTX-M-32}$, carried in *Escherichia coli* strains collected from a cohort of steers housed on a U.S. cattle research-dedicated feedlot in 2009. *bla*$_{CTX-M}$-positive isolates were discovered during investigation of antimicrobial resistance in commingling steers receiving injectable long-acting ceftiofur and/or chlortetracycline in feed regimens. Fecal samples were collected from 88 steers in a 26-day period, and *E. coli* was isolated on MacConkey agar without antibiotics. *bla*$_{CTX-M}$ was detected in *E. coli* isolates from 29/88 steers over the course of the study using the PCR primers CTX-M-F-CCGCTGCCGGTYT TATC and CTX-M-R-ATGTGCAGYACCAGTAA. Of these 29 steers, 14 were treated with ceftiofur, four received chlortetracycline, and 11 received both treatments. Sequencing showed that *E. coli* from each animal carried *bla*$_{CTX-M-32}$ (GenBank accession number AJ557142), a group 1 *bla*$_{CTX-M}$ gene not previously described in U.S. animals but seen throughout Europe in both human and animal isolates (6, 7). A subset of 12 *bla*$_{CTX-M-32}$-positive *E. coli* strains from different steers was selected for further investigation. Pulsed-field gel electrophoresis (8) revealed six distinct *E. coli* strains and seven of the 12 *E. coli* strains with identical banding patterns, indicating that dissemination of *bla*$_{CTX-M-32}$ may therefore be the result of both a clonal expansion and horizontal gene transfer. To investigate plasmid vectors, plasmid DNA from each of the 12 isolates was extracted using a Qiagen plasmid midi kit and transformed into naïve DH10B cells, and *bla*$_{CTX-M-32}$-carrying plasmids were successfully selected on agar containing 2 μg/ml cefotaxime. Plasmids were shown to be self-transmissible to *E. coli* recipient 711 (nalidixic acid resistant [NAL^r]) and conferred no additional antibiotic resistance to the bacterial host. PCR-based replicon typing (9) and plasmid multilocus sequence typing (pMLST) (10) revealed plasmids to be IncN sequence type 1 (ST1), a plasmid group responsible for the extensive spread of group 1 *bla*$_{CTX-M}$ variants in humans and livestock in Europe (10–12).

To our knowledge, this is the first report of *bla*$_{CTX-M-32}$ in isolates from animals in the United States. Furthermore, *bla*$_{CTX-M}$-positive bacteria were found in feces in greater abundance than previously reported (4, 5), even without use of selective antibiotic enrichment. While steers may have acquired *bla*$_{CTX-M-32}$-carrying *E. coli* before entering the feedlot, our data strongly suggest that both spread of an *E. coli* clone between animals and horizontal transfer of an IncN plasmid contributed to wide dissemination of *bla*$_{CTX-M-32}$ among this cohort of steers.

It is important to note that as these isolates were from a single-source steer cohort, the prevalence of *bla*$_{CTX-M}$-carrying strains detailed herein is not representative of the burden across all U.S. feedlots. However, our findings provide salient insights that inform our understanding of the extent to which *bla*$_{CTX-M}$ genes, their plasmid vectors, and bacterial hosts may spread among commingled animals administered a long-acting cephalosporin. These data should be used to inform surveillance activities to better understand the extent of ESBL prevalence in U.S. livestock and to evaluate control strategies that will prevent their widespread dissemination as observed in Europe and Asia.

ACKNOWLEDGMENTS

This study was funded in part by the United States Department of Agriculture, the Cooperative Research Education and Extension Service (now the National Institute of Food and Agriculture), the National Research Initiative (contract number 2008-35201-30235), and the National Integrated Food Safety Initiative (grant number 2010-51110-21083).

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Published ahead of print 19 November 2012
Address correspondence to Patrick Boerlin, pboerlin@uoguelph.ca.

doi:10.1128/AAC.01750-12

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