Whole-Genome Sequence Analysis of CTX-M Containing *Escherichia coli* Isolates from Retail Meats and Cattle in the United States

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In recent years, there have been increased reports on the detection of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Salmonella* strains from food-producing animals and animal products in the United States. We characterized 18 ESBL *E. coli* isolates from cattle (*n* = 5), chicken breast (*n* = 5), ground turkey (*n* = 6), ground beef (*n* = 1), and pork chops (*n* = 1) that were collected by the National Antimicrobial Resistance Monitoring System (NARMS) between 2011 and 2015. *In vitro* antimicrobial susceptibility testing was done against a panel of 14 antimicrobials followed by a secondary panel of 9 β-lactam agents. Whole-genome sequencing was used to characterize the resistome, plasmids, and the genetic structures of the ESBL genes. All ESBL-producing *E. coli* isolates were resistant to at least three antimicrobial classes and carried various *bla* *CTX-M* genes. Most of the cattle and ground turkey isolates carried *bla* *CTX-M-27*. In chicken breast isolates, *bla* *CTX-M-1* was present as part of an IS *Ecp1* transposition unit carried on a plasmid that shares sequence similarity with the backbone structure of the IncI plasmid. Isolates carrying the *bla* *CTX-M-14* and *bla* *CTX-M-15* genes, widely distributed in human clinical isolates, were also isolated. To our knowledge, this is the first report of the widely distributed *bla* *CTX-M-14* and *bla* *CTX-M-15* in *E. coli* isolates from retail meat samples in the United States. Different insertional sequences were identified upstream of these *bla* *CTX-M*s, including IS *Ecp1*, IS26, and IS903-D. CTX-M in *E. coli* from food animals and retail chicken breast were often present on plasmids with other resistance genes. Other resistance genes identified included *aadA*, *strA*, *strB*, *aac(3)-IId*, *aac(3)-Vla*, *aph(3')-Ic*, *blaTEM*, *blaHERA-3*, *floR*, *sul1*, *sul2*, *catA1*, *tetA*, *tetB*, *dfrA*, and *qacE*. These data describe the emergence of CTX-M-carrying *E. coli* isolates in food animals and animal products monitored by NARMS program.

Keywords: *Escherichia coli*, ESBL, CTX-M

Introduction

Extended-spectrum beta-lactamases (ESBLs) are the most common cephalosporin resistance mechanism reported in members of the *Enterobacteriaceae* family. ESBLs are a group of enzymes with the ability to hydrolyze oxyimino-cephalosporins and thus cause resistance to ceftaxime, cefotaxime, ceftriaxone, cefuroxime, and cefepime, as well as monobactams (*e.g.*, aztreonam). The introduction of extended-spectrum cephalosporins in clinical practice in the 1970s was soon followed by reports of resistant strains of Enterobacteriaceae producing ESBLs. Since then, the occurrence of infection due to ESBL-resistant Enterobacteriaceae has rapidly increased and has become a major problem worldwide. In the United States, the Centers for Disease Control and Prevention estimates 26,000 infections and 1,700 deaths annually due to ESBL-producing Enterobacteriaceae. CTX-M-producing *Escherichia coli* have been associated with both hospital-acquired and community infections, mostly in urinary tract infections and bacteremia. Studies over the last decade have shown that CTX-M-type ESBLs...
have become the predominant enzyme type in many parts of the world,\textsuperscript{9} and have spread rapidly through clinical populations of Enterobacteriaceae.\textsuperscript{10} Reports from several countries describe the presence of CTX-M-producing \emph{E. coli} strains in apparently healthy food animals\textsuperscript{11–13} and food animal products,\textsuperscript{12} as well as pets\textsuperscript{13} and wild birds.\textsuperscript{14} Although CTX-M-producing strains appear to have quickly spread worldwide, the increased prevalence of \emph{E. coli} carrying these \beta-lactamases in numerous U.S. hospitals became apparent in the early 2000s.\textsuperscript{7,10,16}

Intestinal carriage of CTX-M-producing bacteria in food-producing animals and contamination of retail meat might contribute to increased occurrences of infections with ESBL-producing bacteria in humans. A study on the presence of indistinguishable \emph{E. coli} genotypes carrying CTX-M genes obtained from poultry, poultry products, and human clinical samples in the Netherlands has suggested the possible exchange of these genes through the food chain.\textsuperscript{12} In the United States, few CTX-M ESBLs have been reported from food animals and animal products.\textsuperscript{11,17–19} The National Antimicrobial Resistance Monitoring System (NARMS) monitors changes in antimicrobial susceptibilities of zoonotic foodborne bacteria to medically important antimicrobials, including \beta-lactam antibiotics. Whole-genome sequencing has improved our ability to monitor resistomes and helps to identify and characterize emerging resistance genes and mobile genetic elements that facilitate the spread of these genes. The aim of this study was to investigate and characterize antimicrobial resistance genes and mobile genetic elements associated with phenotypically positive ESBL \emph{E. coli} isolates recovered from cattle and retail meat samples collected through the NARMS program between 2011 and 2015. This information will help to characterize the molecular epidemiology of CTX-M carrying \emph{E. coli} isolates in food animals and animal products monitored by the NARMS program.

**Materials and Methods**

**Bacterial strains**

Eighteen phenotypically positive ESBL \emph{E. coli} isolates recovered from cattle fecal samples \((n=5)\) and retail meats (chicken breast \([n=5]\), ground turkey \([n=6]\), ground beef \([n=1]\), and pork chops \([n=1]\)) by the NARMS program between 2011 and 2015 were identified and selected for characterization. The isolates were identified from a total of 8,721 \emph{E. coli} isolates recovered from fecal samples of healthy cattle and retail meat samples. The fecal isolates \((n=3,079)\) were recovered from healthy cattle as part of a NARMS on-farm pilot program to monitor antimicrobial resistance in foodborne pathogens. The retail meat \emph{E. coli} isolates \((n=5,642)\) were recovered from chicken breast, chicken wing, pork chops, ground beef, and ground turkey.

**In vitro antimicrobial susceptibility testing**

The bacterial isolates were tested for antimicrobial drug susceptibility using the Sensititre\textsuperscript{\textregistered} semiautomated antimicrobial susceptibility system (ThermoFisher Scientific, Trek Diagnostics, Cleveland, OH) following the manufacturer’s instructions. The antimicrobials tested were as follows: amoxicillin/clavulanic acid (AUG), ampicillin (AMP), azithromycin (AZM), cefoxitin (FOX), cefotiofur (TIO), ceftriaxone (CRO), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), nalidixic acid (NAL), streptomycin (STR), sulfamethoxazole (SMX), tetracycline (TET), and trimethoprim/sulfamethoxazole (SXT).

A total 321 \emph{E. coli} isolates with minimum inhibitory concentrations (MICs) \(\geq 8 \mu\text{g/mL}\) for cefotiofur and/or \(\geq 4 \mu\text{g/mL}\) for ceftriaxone for isolates recovered before 2015 and \(\geq 2 \mu\text{g/mL}\) for isolates recovered in 2015 were selected for further testing with a second panel of 9 \beta-lactam antimicrobials: aztreonam (ATM), cefquinome (CQN), imipenem (IMI), ceftazidime (TAZ), ceftazidime–clavulanic acid (CAZ/CLA), cefotaxime (FOT), and cefotaxime–clavulanic acid (CTX/CLA). The Clinical and Laboratory Standards Institute (CLSI) confirmatory test for ESBL production was used and is based on cefotaxime and cefazidime MICs with and without clavulanic acid. Isolates showing a three or more twofold concentration decrease in the cefotaxime and cefazidime MICs when tested in combination with clavulanate versus the MICs of cefotaxime and cefazidime when tested alone are considered ESBL\textsuperscript{\textregistered}.\textsuperscript{20}

\emph{E. coli} ATCC 25922, \emph{Enterococcus faecalis} ATCC 29212, \emph{Staphylococcus aureus} ATCC 29213, \emph{Pseudomonas aeruginosa} ATCC 27853, and \emph{Klebsiella pneumoniae} ATCC 700603 were used as quality control organisms for MIC determinations. Results were interpreted according to CLSI guidelines for broth microdilution methods with the exception of STR (NARMS resistance breakpoint, \(\geq 32 \mu\text{g/mL}\)), AZM (NARMS resistance breakpoint, \(\geq 32 \mu\text{g/mL}\)), and CQN (NARMS resistance breakpoint, \(\geq 32 \mu\text{g/mL}\)).\textsuperscript{20}

**Conjugation**

Conjugation experiments using a plate mating protocol were used to determine the transferability of resistance phenotypes and localize CTX-M genes to conjugative plasmids. We selected seven \emph{E. coli} isolates that carried different CTX-M genes \((N36254PS (bla_{CTX-M-32}), N36410PS (bla_{CTX-M-32}), N37058PS (bla_{CTX-M-32}), N40513 (bla_{CTX-M-1}), N40607 (bla_{CTX-M-1}), N46045 (bla_{CTX-M-15}), and N51980 (bla_{CTX-M-14})) as donor cells. MAX Efficiency\textsuperscript{\textregistered} DH5\textsuperscript{\textregistered} \emph{E. coli} Competent Cells (Invitrogen, Carlsbad, CA) were used as recipients. The donors and recipients were grown in 2 mL LB medium (Becton Dickinson, Sparks, MD) at 37\textdegree C in a shaker incubator for 16–18 hours. Ten microliters of donor cells were spotted on top of 10 \muL of recipient strain (DH5\textsuperscript{\textregistered}) on blood agar plates and incubated at 37\textdegree C overnight. Each co-culture was then scraped from the plate and resuspended in 1 mL LB broth. Cefotiofur and nalidixic acid (Sigma-Aldrich, St. Louis, MO) were used as selective agents for the donor and recipient strains, respectively. Transconjugants were selected on LB agar containing nalidixic acid (30 \mug/mL) and cefotiofur (4 \mug/mL). The MICs of donors, recipients, and transconjugants were determined using the Sensititre semiautomated antimicrobial susceptibility system. The \beta-lactam susceptibility testing panel was used to confirm the phenotype.

Five transconjugants from each experiment were tested for the presence of CTX-M genes using PCR primers designed for CTX-M-1 group (forward primer, 5’-ATGGTTA AAAAATCACTGCGTCAGT-3’, reverse primer, 5’-TTCACACCCGTTGTGAGAATTAGCC-3’) and CTX-M-9 group (forward primer, 5’-ATGTTGAACAAAGAGAGTCG
AACGG-3' reverse primer, 5'-TTACAGCGCTTGGCGCA
TGATTCT-3'). The expected amplicon fragment size
for CTX-M group 1 and 9 was 876 and 846 bp, respectively.
The PCR amplification conditions included initial denaturation
at 95°C for 10 minutes, 30 cycles of denaturing at 94°C for 30
seconds, annealing at 58°C for 60 seconds, and extension at
72°C for 60 seconds, and followed by final extension at 72°C
for 7 minutes.

**PCR-based plasmid replicon typing of transconjugants**

Genomic DNA was extracted using the DNeasy Blood
and Tissue kit (Qiagen, Valencia, CA) following the
manufacturer’s instructions. Amplification of plasmid replicon
targets was carried out following the protocol de-
scribed by Johnson et al.21 with minor modifications for
IncP characterization. For IncP, a simplex PCR with an
annealing temperature of 65°C was used. The amplified
products were separated by gel electrophoresis on 1.0%
agarose gels.

**Whole-genome sequencing**

Whole-genome sequencing was used to characterize the
resistome and plasmids in all strains (n = 18). Briefly, DNA
was extracted using the DNeasy Blood and Tissue Kit (Qiagen)
following the manufacturer’s instructions. Whole-
genome sequencing was performed on the MiSeq Desktop
Sequencer using v2 sequencing reagent kits (Illumina, San
Diego, CA). A de novo assembly was performed using CLC
Genomics Workbench version 8.0 (Qiagen). Contigs of less
than 200 bp were removed from analysis. The number of
assembled contigs ranged between 78 and 258 with an
average coverage of 50×.

**Resistome analysis**

Resistance genes were identified using BLASTXa and the
ResFinder resistance gene database.22 The BLASTX results
were processed with in-house PERL scripts to identify an-
timicrobial resistance genes using an 85% amino acid
identity and 50% minimum sequence length.

**Phylogenetic analysis**

The Center for Food Safety and Applied Nutrition
(CFSAN) SNP pipelineb was used to create the single nu-
cleotide polymorphism (SNP) matrices from sequence data
for the phylogenetic analysis. SNP redundancy by linkage
disequilibrium was reduced and the phylogenetic tree was
constructed with the maximum likelihood algorithm using
the SnpPhylo package.23

**Plasmid profiling**

Identification of the plasmid type was done using the
PlasmidFinder database.2 The cutoff threshold for identity
was set at 95% to determine the existence for a particular
plasmid.

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Multilocus sequence typing profiling

*E. coli* multilocus sequence typing (MLST) allelic pro-
files and sequences were downloaded from the PubMLST
database.2 A total of 7,113 profiles for 7 different loci were
used for the MLST. The SRST2 pipeline22 was used to
determine the MLST type for our *E. coli* isolates.

**Nucleotide sequence accession numbers**

The whole-genome sequence data reported in this study
have been deposited at DDBJ/ENA/GenBank under the
following accession numbers: AZCE00000000, AZCG0000
0000, AZCB00000000, AZCC00000000, AZCD00000000,
AZCF00000000, AZCH00000000, NTSM00000000, NT
MT00000000, NTMU00000000, NTV00000000, NTMY
000000, NTMX00000000, NTMY00000000, NTMZ00000
00, NTNA00000000, NTNB00000000, and NTNC00000000.

**Results**

In vitro antimicrobial susceptibility testing
of *E. coli* isolates

The antimicrobial resistance profiles of phenotypically
positive ESBL *E. coli* isolates are shown in Table 1. All the
isolates were resistant to ampicillin, ceftiofur, ceftriaxone,
and cefotaxime, as expected. Three of the 5 cattle and 2 of
the 13 retail meat *E. coli* isolates were resistant to ceftoxi-
nome, a fourth-generation cephalosporin. In addition, one of
the cattle *E. coli* isolates and two of retail meat isolates
showed resistance to aztreonam, a monobactam. Among the
18 strains producing ESBL, all had a three or more twofold
concentration decrease in MIC for cefotaxime and ceftazi-
dime in combination with clavulanic acid than the MIC
when tested alone (Table 2). Other non-β-lactam resistances
observed were to sulfisoxazole (15/18), tetracycline (14/18),
chloramphenicol (3/18) streptomycin (9/18), nalidixic acid
(1/18), and trimethoprim/sulfamethoxazole (4/18).

**Conjugation and plasmid typing**

At least two plasmid types were detected using Plas-
midFinder from each of the *blaCTX-M + E. coli* isolates
(Fig. 1). The conjugation results showed that *blaCTX-M+
* genes can be transferred by broth mating. The transfer of
*blaCTX-M* gene was confirmed by PCR. We further con-
formed the plasmid replicon type of the transconjugant with
the ESBL phenotype using PCR-based replicon typing.
Based on the replicon typing, the two conjugative plasmid
types that carried the CTX-M genes were IncI1 and IncF,
present in isolates from chicken breast and cattle feces, re-
spectively. One of the *blaCTX-M* genes identified from cattle
isolates (*blaCTX-M-32*) was not transferable by conjugation.
Other resistance phenotypes co-transferred by conjugation
include TetK and SmxK (Table 1).

**Resistome analysis in ESBL *E. coli* isolates**

Characterization of resistance genes was conducted using
whole-genome sequencing. The distribution of resistance
genes is shown in Fig. 2. The ESBL genotypes in our

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a[https://blast.ncbi.nlm.nih.gov/](https://blast.ncbi.nlm.nih.gov/)
b[http://snp-pipeline.readthedocs.io/](http://snp-pipeline.readthedocs.io/)
c[https://cge.cbs.dtu.dk/services/PlasmidFinder](https://cge.cbs.dtu.dk/services/PlasmidFinder)
d[https://pubmlst.org/](https://pubmlst.org/)
| Antimicrobials<sup>a</sup> | AMC (≥32/16) | AMP (≥32) | AZM<sup>b</sup> (≥32) | FOX (≥24) | CRO (≥24) | MEM (≥4) | CHL (≥32) | CIP (≥4) | NAL (≥16) | GEN (≥16) | STR<sup>b</sup> (≥32) | TET (≥16) | FIS (≥16) | SXT (≥4/76) |
|---------------------------|--------------|------------|-----------------|----------|----------|----------|----------|----------|----------|-----------|--------------|-----------|----------|-------------|
| N36254PS Farm, fecal      | 4 >32        | 4 8        | 8 ≤0.06         | 8 ≤0.015 | 2 0.5     | 32 >32   | 256 ≤0.12|          |          |           |              |           |          |              |
| N36410PS Farm, fecal      | 8 >32        | 4 8 >64    | 8 ≤0.06         | 8 ≤0.015 | 2 0.5     | 16 ≤4    | 256 >4   |          |          |           |              |           |          |              |
| N37058PS Farm, fecal      | 4 >32        | 4 4 32     | ≤0.06 >32       | 1 4       | 0.5      | 32 >32   | 256 ≤0.25|          |          |           |              |           |          |              |
| N37122PS Farm, fecal      | 4 >32        | 4 8 64     | 8 ≤0.06         | 8 ≤0.015 | 2 1       | 8 ≤4     | 256 >4   |          |          |           |              |           |          |              |
| N37139PS Farm, fecal      | 4 >32        | 4 8 64     | 8 ≤0.06         | 8 ≤0.015 | 2 0.5     | 8 ≤4     | 256 >4   |          |          |           |              |           |          |              |
| N40513 Chicken breast     | 8 >32        | 4 4 64     | 8 ≤0.06         | 4 ≤0.015 | 2 0.5     | 8 ≤4     | 256 >4   |          |          |           |              |           |          |              |
| N40607 Chicken breast     | 8 >32        | 2 8 >64    | 8 ≤0.06         | 4 ≤0.015 | 4 0.5     | 16 >32   | 256 >4   |          |          |           |              |           |          |              |
| N43684 Chicken breast     | 8 >32        | 2 2 >64    | 8 ≤0.06         | 6 ≤0.015 | 2 0.5     | 8 ≤4     | 256 >4   |          |          |           |              |           |          |              |
| N44807 Ground turkey      | 8 >32        | 4 8 >64    | 8 ≤0.06         | 8 ≤0.015 | 4 ≥25 >64 | 32 ≤16   | 0.12 ≤0.12|          |          |           |              |           |          |              |
| N56041 Ground turkey      | 32 >32       | 4 8 >64    | 8 ≤0.06         | 32 ≤0.15 | 4 0.5     | ≥64 >32  | 256 ≤0.12|          |          |           |              |           |          |              |
| N51980 Pork chop           | 8 >32        | 4 4 64     | 8 ≤0.06         | 8 ≤0.015 | 2 0.5     | >64 ≥32  | ≥16 ≤0.12|          |          |           |              |           |          |              |
| N60592 Ground turkey      | 8 >32        | 4 4 64     | 8 ≤0.06         | 4 ≤0.15  | 2 0.5     | 8 ≤4     | 256 >4   |          |          |           |              |           |          |              |

<sup>a</sup>CLSI breakpoint.
<sup>b</sup>NRAMS breakpoint.

AMC, amoxicillin/clavulanic acid; AMP, ampicillin; AZM, azithromycin; CHL, chloramphenicol; CIP, ciprofloxacin; CRO, ceftriaxone; FIS, sulfisoxazole; FOX, ceftoxin; GEN, gentamicin; MEM, meropenem; NAL, nalidixic acid; STR, streptomycin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline.

### Table 2. Minimum Inhibitory Concentration Values from the Extended-Spectrum Beta-Lactamase Panel for *Escherichia coli* Isolates Obtained from Fecal Samples of Healthy Cattle and Retail Meat Samples with Diminished Susceptibility or Resistance to Broad-Spectrum Cephalosporins

| Source          | ATM (≥16) | FEP (≥16) | CTX (≥16) | CQN<sup>b</sup> (≥32) | CAZ (≥16) | IPM (≥16) | TZP (≥128/4) | CTX/CLA | CAZ/CLA |
|-----------------|----------|-----------|----------|-------------------------|-----------|-----------|--------------|---------|---------|
| N36254PS Farm, fecal | 2 1 8 4 | 1 0.12 ≤0.5 | ≤0.06 | ≤0.06 |          |          |              | —       | —       |
| N36410PS Farm, fecal | 16 4 64 >32 4 | 0.12 | 8 0.25 | 0.12 |          |          |              | —       | —       |
| N37058PS Farm, fecal | 8 4 32 16 4 | 0.25 | 1 ≤0.06 | ≤0.06 |          |          |              | —       | —       |
| N37122PS Farm, fecal | 8 8 128 32 2 | 0.12 | 1 ≤0.06 | ≤0.06 |          |          |              | —       | —       |
| N37139PS Farm, fecal | 8 4 64 32 8 | 0.12 ≤0.5 | ≤0.06 | ≤0.06 |          |          |              | —       | —       |
| N40513 Chicken breast | 8 4 16 8 1 | 0.25 | 1 ≤0.06 | ≤0.06 |          |          |              | —       | —       |
| N40607 Chicken breast | 16 8 64 16 8 | 0.25 | 2 ≤0.06 | 0.25 |          |          |              | —       | —       |
| N43684 Chicken breast | >32 16 128 >32 8 | 0.25 | 2 ≤0.12 | 0.25 |          |          |              | —       | —       |
| N44807 Ground turkey | 8 4 64 16 1 | 0.25 | 2 ≤0.12 | 0.25 |          |          |              | —       | —       |
| N46045 Ground beef | 4 8 32 32 1 | 0.12 | 1 ≤0.06 | 0.25 |          |          |              | —       | —       |
| N51980 Pork chop | 4 2 32 8 4 | 0.12 | 2 ≤0.06 | 0.12 |          |          |              | —       | —       |
| N53976 Ground turkey | 8 8 32 16 1 | 0.25 | 2 ≤0.06 | 0.12 |          |          |              | —       | —       |
| N56041 Ground turkey | 4 4 16 16 2 | 0.25 | 2 ≤0.06 | 0.25 |          |          |              | —       | —       |
| N56738 Ground turkey | 4 4 32 16 4 | 0.12 | 2 ≤0.06 | 0.12 |          |          |              | —       | —       |
| N58201 Chicken breast | 4 2 16 8 2 | 0.12 | 2 ≤0.06 | 0.25 |          |          |              | —       | —       |
| N60559 Chicken breast | 4 4 16 8 1 | 0.25 | 1 ≤0.06 | 0.25 |          |          |              | —       | —       |
| N60592 Ground turkey | 8 8 32 16 2 | 0.12 | 1 ≤0.06 | 0.25 |          |          |              | —       | —       |
| N63148 Ground turkey | 8 2 16 8 8 | 0.12 | 4 8 |          |          |              | —       | —       |

<sup>a</sup>CLSI breakpoint.
<sup>b</sup>NRAMS breakpoint.

ATM, aztreonam; CAZ, ceftazidime; CAZ/CLA, ceftazidime–clavulanic acid; CQN, cefquinome; CTX, cefotaxime; CTX/CLA, cefotaxime–clavulanic acid; FEP, cephepine; IPM, imipenem; TZP, piperacillin–tazobactam.
isolates were \textit{bla}\textsubscript{CTX-M-1}, \textit{bla}\textsubscript{CTX-M-14}, \textit{bla}\textsubscript{CTX-M-15}, \textit{bla}\textsubscript{CTX-M-27}, and \textit{bla}\textsubscript{CTX-M-32}. CTX-M \( \beta \)-lactamases exhibit increased hydrolytic activity against cefotaxime and ceftriaxone, but generally not against ceftazidime, which has important implications for laboratory detection. Similarly, in this study, all CTX-M-positive isolates were resistant to cefotaxime and none were resistant to ceftazidime. This is the first report of \textit{bla}\textsubscript{CTX-M-14} and \textit{bla}\textsubscript{CTX-M-15}-containing \textit{E. coli} isolates from NARMS retail meat program in the United States.

The CTX-M genes identified in our isolates had 100% nucleotide sequence identity with previously reported CTX-M genes. All five of the chicken breast \textit{E. coli} isolates carried \textit{bla}\textsubscript{CTX-M-1}, four of the five cattle isolates (N36410PS, N37058PS, N37122PS, and N37139PS) had \textit{bla}\textsubscript{CTX-M-27}, and one cattle isolate (N36254PS) contained \textit{bla}\textsubscript{CTX-M-32}.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Plasmid and MLST profiling of \textit{bla}\textsubscript{CTX-M}-encoding \textit{Escherichia coli} isolates. Phylogenetic tree generated based on the SNP analysis of the WGS data. The \textit{black} color indicates the presence of the plasmid type based on plasmid finder. \textit{6707*}—a single SNP on recA (T176G) and 2325*—a single SNP on purA (G102A). MLST, multilocus sequence typing; SNP, single nucleotide polymorphism.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Phylogenetic analysis and summary of MLST profiling and antimicrobial resistance genes identified in phenotypically positive ESBL \textit{E. coli} isolates recovered from cattle and chicken breast. Clustering based on the SNP analysis of WGS data. The \textit{black} color indicates the resistance gene identified. ESBL, extended-spectrum beta-lactamase.}
\end{figure}

\footnote{https://cge.cbs.dtu.dk/services/PlasmidFinder}
FIG. 3. Genetic context of insertion sequence elements (ISEp1, IS903D, and IS26) found in association with $bla_{CTX-M}$ genes. CTX-M genes are indicated with red arrows. Insertion sequences and transposons are represented with blue arrows.
Three of the four \( \text{bla}_{\text{CTX-M-27}} \) + cattle \( \text{E. coli} \) isolates clustered together and had identical MLST type (ST1508) (Fig. 2).

\( \text{E. coli} \) isolates recovered from ground turkey carried diverse CTX-M genes, including \( \text{bla}_{\text{CTX-M-1}} \) (\( n = 1 \)), \( \text{bla}_{\text{CTX-M-15}} \) (\( n = 1 \)), and \( \text{bla}_{\text{CTX-M-27}} \) (\( n = 4 \)). A single \( \text{E. coli} \) isolate each from pork chops and ground beef carried \( \text{bla}_{\text{CTX-M-14}} \) and \( \text{bla}_{\text{CTX-M-15}} \), respectively. The primary mechanisms responsible for the acquisition and mobilization of CTX-M genes are insertions sequences, transposons, and ISCR1.

In our isolates, we identified \( \text{ISEcp1} \), IS26, and IS903-D mobilization elements (Fig. 3). In chicken breast isolates, \( \text{bla}_{\text{CTX-M-1}} \) gene was present as part of an \( \text{ISEcp1} \) transposition unit and shares sequence similarity with the backbone structure of the IncI plasmid. Conjugation results demonstrated that \( \text{tet} \) and \( \text{sul} \) resistance genes were carried on the same IncI plasmids harboring \( \text{bla}_{\text{CTX-M-1}} \) gene.

Streptomycin-resistant isolates (MIC \( \geq 32 \)) carried one or more aminoglycoside resistance genes. The aminoglycoside resistance genes commonly identified in these isolates were \( \text{strA} \) [\( \text{aphI}^3 \)-IIb], \( \text{strB} \) [\( \text{aphI}^6 \)-IId], and \( \text{aadA1} \). One isolate with a gentamicin MIC >\( 16 \) \( \mu \)g/mL possessed an \( \text{aacI}^3 \)-IId gene. Three \( \text{E. coli} \) isolates (N36410PS, N37122PS, and N37139PS) contained a class I integron carrying \( \text{aadA1} \), \( \text{dfrA} \), and \( \text{qacE} \) genes.

Two chloramphenicol-resistant isolates carried the \( \text{floR} \) gene and one contained \( \text{catA1} \). All, but three \( \text{E. coli} \) isolates were resistant to sulfoxazole and had either \( \text{sul1} \) or \( \text{sul2} \). Four also carried the dihydrofolate reductase gene \( \text{dfrA} \).

**Discussion**

CTX-M-producing strains appear to have quickly spread worldwide, with the notable exception of the United States where TEM- and SHV-type ESBL have appeared to predominate until recently. CTX-M ESBLs have been reported in the United States mainly from human clinical isolates of Enterobacteriaceae encoding for CTX-M group 1 and 9.7,10,16, 25,26 Infections caused by bacteria producing CTX-M enzymes are not limited to the hospital setting.27 Intestinal carriage of CTX-M-producing bacteria in food-producing animals and contamination of retail meat may contribute to increased incidences of infections with ESBL-producing bacteria in humans. Various reports have documented dissemination of ESBL-producing \( \text{E. coli} \) in healthy food-producing animals and animal products in several countries,12,13,17,28,29 and the potential of wild birds as possible reservoirs and vehicles for dissemination of CTX-Ms in the United States.15

In this study, we are reporting the first \( \text{bla}_{\text{CTX-M-14}} \) and \( \text{bla}_{\text{CTX-M-15}} \) gene carrying \( \text{E. coli} \) isolate from NARMS retail meat program. All \( \text{E. coli} \) isolates obtained before 2011 from NARMS were phenotypically and genotypically negative for \( \text{bla}_{\text{CTX-M-1}} \).30 However, McDermott et al. recently identified the first \( \text{bla}_{\text{CTX-M-1}} \)-positive Salmonella isolate recovered from NARMS retail meat samples in the United States.19 Salmonella enterica serovar Infantis isolates containing \( \text{bla}_{\text{CTX-M-65}} \) obtained from chicken, cattle, and human sources collected between 2012 and 2015 in the United States through routine NARMS surveillance have been reported.19 Davis et al., reported \( \text{bla}_{\text{CTX-M}} \)-carrying \( \text{E. coli} \) strains among isolates collected from Washington State cattle in 2011, while none from those collected in 2008.13 Investigations of nontyphoidal Salmonella isolates of human origin submitted to Center for Disease Control and Prevention (CDC) as part of the NARMS program between 2005 and 2007 identified Salmonella isolates producing CTX-M enzymes (\( \text{bla}_{\text{CTX-M-15}}, \text{ bla}_{\text{CTX-M-5}}\), and \( \text{bla}_{\text{CTX-M-55S57}} \)).

The successful spread of CTX-M genes depends on the clonal nature of strains carrying the resistance genes, and mobile genetic elements responsible for its capture and spread. \( \text{E. coli} \) ST131 and ST405 are by far the most important sequence types (STs) associated with the spread of CTX-M genes, including \( \text{bla}_{\text{CTX-M-15}} \) and other CTX-M genes such as \( \text{bla}_{\text{CTX-M-1}}, \text{ bla}_{\text{CTX-M-3}}, \text{ bla}_{\text{CTX-M-10}}, \) and \( \text{bla}_{\text{CTX-M-14}}. \) None of our \( \text{bla}_{\text{CTX-M}} \) \( \text{E. coli} \) belonged to ST131 or ST405, indicating that the spread of CTX-M genes is not associated with the established clonal strains. We observed diverse STs carrying the same \( \text{bla}_{\text{CTX-M-1}} \) gene, and in some instances, different \( \text{bla}_{\text{CTX-M}} \) genes carried by the same ST. For example, \( \text{E. coli} \) isolates recovered from two ground turkey isolates carrying \( \text{bla}_{\text{CTX-M-27}} \) and two chicken breast isolates carrying \( \text{bla}_{\text{CTX-M-1}} \) were ST117, indicating the potential of the same ST to spread multiple CTX-M genes.

The most commonly reported mobilization elements mediating the spread of CTX-M genes include \( \text{ISEcp1} \), ISCR1, and IS264–38 and phage-related sequences.37 In our isolates, different IS elements, including \( \text{ISEcp1} \), IS26, and IS903-D were identified upstream of \( \text{bla}_{\text{CTX-M-1}} \), \( \text{bla}_{\text{CTX-M-27}} \), and \( \text{bla}_{\text{CTX-M-32}} \) genes as previously reported elsewhere.38–40 All phenotypically positive ESBL \( \text{E. coli} \) isolates recovered from chicken breast encoded \( \text{bla}_{\text{CTX-M-1}} \) as part of an \( \text{ISEcp1} \) transposition unit and shares sequence similarity with the backbone structure of the IncI plasmid. IncI1 has been shown to be one of the main plasmid lineages that contribute to the dissemination of \( \text{bla}_{\text{CTX-M-1}} \) genes in the food chain, including chicken retail meat, the environment, and humans.41 In the Netherlands, bacteria producing ESBL isolated from chicken meat and gut of broilers predominantly carried \( \text{bla}_{\text{CTX-M-1}} \) located on IncI1 plasmids.42 Similarly, Day et al. demonstrated a widespread distribution of IncI1 plasmids carrying \( \text{bla}_{\text{CTX-M-1}} \) gene among \( \text{E. coli} \) recovered from humans, animals, and food products in Germany, the Netherlands, and the United Kingdom.43 Furthermore, a study in the Netherlands revealed the presence of indistinguishable genotypes, CTX-M genes and plasmids, in \( \text{E. coli} \) obtained from poultry, retail chicken meat, and human clinical samples, suggesting possible exchange through food chain.42

The \( \text{bla}_{\text{CTX-M-27}} \) genes identified in our cattle and ground turkey \( \text{E. coli} \) isolates were associated with IncF plasmids. Horizontal transfer is important in the dissemination of \( \text{bla}_{\text{CTX-M-27}} \) gene, as evidenced by the fact that \( \text{bla}_{\text{CTX-M-27}} \) genes in our isolates were transferred by conjugation, confirming the location of the gene on a conjugative plasmid. IncF plasmids encode numerous addiction systems that ensure and contribute to the maintenance of antimicrobial resistance determinants and virulence factors even in the absence of antibiotic selection pressure.44 IncF replicon-type plasmids carrying \( \text{bla}_{\text{CTX-M-27}} \) have been documented in cefotaxime-producing \( \text{E. coli} \) clinical isolates from Dublin, Ireland.45 Plasmids carrying \( \text{bla}_{\text{CTX-M}} \) genes are often self-conjugative and carry additional resistance determinants,46 greatly facilitating widespread distribution of alleles in different environments. A recent report has documented a case of ceftriaxone treatment failure caused by Salmonella Typhimurium due to
the in vivo acquisition of a $bla_{CTX-M-27}$-encoding IncFII group transmissible plasmid.\textsuperscript{47}

The presence of $bla_{CTX-M-15}$ and $bla_{CTX-M-14}$ was reported in most U.S. medical centers participating in the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) program in 2007.\textsuperscript{10,25} In this study, we are reporting for the first time $bla_{CTX-M-15}$ and $bla_{CTX-M-14}$-encoding E. coli from retail meat samples collected in 2013. CTX-M-15 and CTX-M-14, the two most frequently identified CTX-M enzyme worldwide, have been detected in bacteria isolated from humans, animals, and the environment.\textsuperscript{17,42,48} A recent study from six community hospitals in North Carolina and Virginia from 2010 to 2012 demonstrated that 80% of ESBL-producing isolates contained CTX-M enzymes. In these isolates, ST131 was associated with 48% of $bla_{CTX-M-15}$-producing E. coli isolates and 66% of the $bla_{CTX-M-14}$-producing E. coli isolates.\textsuperscript{49} While the prevalence of these two successful CTX-M enzymes is low from domestic food animal sources, monitoring will continue to help determine whether this mechanism is becoming more widespread among animal and food strains of E. coli in the United States.

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Disclosure Statement

G.H.L. has accepted consulting fees for service on scientific advisory boards, honoraria, and travel expenses from various manufacturers of animal pharmaceuticals.

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