Occurrence of OXA-48 Carbapenemase and Other β-Lactamase Genes in ESBL-Producing Multidrug Resistant Escherichia coli from Dogs and Cats in the United States, 2009–2013

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Objective: The aim of this study was to explore the occurrence and molecular characterization of extended-spectrum β-lactamases (ESBL), plasmid-mediated AmpC β-lactamase (pAmpC) and carbapenemases among ESBL-producing multidrug resistant (MDR) Escherichia coli from dogs and cats in the United States.

Methods: Of 2443 E.coli isolated from dogs and cats collected between August 2009 and January 2013, 68 isolates were confirmed as ESBL-producing MDR ones. PCR and sequencing were performed to identify β-lactamases and plasmid-mediated quinolone resistance (PMQR) genes, and shed light on the virulence gene profiles, phylogenetic groups and ST types.

Results: Phylogenic group D and B2 accounted for 69.1% of the isolates. 50 (73.5%) isolates carried CTX-M ESBL gene, and the most predominant specific CTX-M subtype identified was blaCTX-M−15 (n = 33), followed by blaCTX-M−1 (n = 32), blaCTX-M−123 (n = 27), blaCTX-M−9 (n = 19) and blaCTX-M−14 (n = 19), and blaCTX-M−123 was firstly reported in E. coli isolates in the United States alone or in association. Other β-lactamase genes blaTEM, blaSHV, blaOXA−48, and blaCMY−2 were detected in 41.2, 29.4, 19.1, and 17.6% of 68 ESBL-producing MDR isolates, respectively. The blaTEM and blaSHV genes were classified as ESBLs with the exception of the blaTEM−1 gene. Additionally, 42.6% (29/68) of isolates co-expressed blaCTX-M−15 and PMQR gene aac(6′)-Ib-c. The overall occurrence of virulence genes ranged from 11.8 (ireA) to 88.2% (malX), and most of virulence genes were less frequent among CTX-M-producing isolates than non-CTX-M isolates with the exception of malX and iutA. The 68 isolates analyzed were assigned to 31 STs with six being novel. Three pandemic clonal lineages ST131 (n = 10), ST648 (n = 9), and ST405 (n = 9) accounted for more than 41% of the investigated isolates, and ST648 and ST405 of phylogenetic D were firstly reported in E. coli from dogs and cats in the United States.
INTRODUCTION

Extraintestinal pathogenic strains of *Escherichia coli* (ExPEC) are the most important dogs and cats bacterial pathogens associated with extraintestinal infections (Beutin, 1999). However, extended-spectrum β-lactamase (ESBL)-producing ExPEC are isolated worldwide with increasing frequency from human and animal clinical isolates (Pitout, 2012). The occurrence of β-lactamases, including ESBLs, plasmid-mediated AmpC β-lactamases (pAmpC) and carbapenemases among *E. coli* pose serious challenges to the use of penicillins, extended-spectrum cephalosporins (3rd and 4th generation cephalosporins), monobactams, and carbapenems (Karisik et al., 2008; Geser et al., 2012). Furthermore, ESBL-producing isolates are often cross-resistant to fluoroquinolones and other antimicrobial agents, thus expressed multidrug resistance (MDR). This combination of properties can significantly affect the course and outcomes of infections. β-lactamase genes commonly located on mobile genetic elements, such as plasmids, transposons, or integrons, and the resistance plasmids can easily be transferred between bacterial isolates by conjugation mechanism. Accordingly, transmission of β-lactamase genes between companion animals and owner has become a subject of active discussion as companion animals could be potential sources of ESBL-producing *E. coli* isolates causing community-acquired infections (Schmiedel et al., 2014).

Although the ESBLs, pAmpCs and carbapenemases in *E. coli* isolates from humans and animals have been characterized in various studies around the world, knowledge about the β-lactamases and population structure in MDR *E. coli* isolates from companion animals in the United States is limited. Prior to the current study only two studies have described the occurrence and the diversity of ESBLs in *E. coli* from dogs and cats in the United States (O’Keefe et al., 2010; Shaheen et al., 2011), and the isolates were collected from September 2004 to December 2007, and May 2008 to May 2009, respectively. However, the β-lactamases, particularly CTX-M-type ESBLs, are characterized by ongoing and complex evolution. Currently, greater than 150 variants have been identified, and several chimeras, e.g., *bla*<sub>CTX-M−64</sub> and *bla*<sub>CTX−M−123</sub> have been reported since 2009 (He et al., 2013). Moreover, several novel β-lactamases, e.g., *bla*<sub>pPC</sub>, *bla*<sub>SDM−1</sub>, and *bla*<sub>OXA−48</sub> are emerging worldwide in *E. coli* isolated from humans or animals.

The aim of the present study was to (i) investigate the occurrence and molecular characterization of ESBL-producing MDR *E. coli* recovered from clinical cases of infection in dogs and cats in the United States, over a period of time ranging from August 2009 to January 2013, and (ii) characterize the association between β-lactamases, phylogenetic groups, virulence genes and the ST types.

**Conclusion:** The *bla*<sub>CTX−M−123</sub> of ESBLs and carbapenemase *bla*<sub>OXA−48</sub> were firstly reported in ESBL-producing MDR *E. coli* from dogs and cats in the United States, and ST131, ST648, and ST405 were the predominant clonal groups.

**Keywords:** *Escherichia coli*, ESBL, OXA-48 carbapenemase, multidrug resistance, companion animals

MATERIALS AND METHODS

**Bacterial Isolates**

Between August 2009 and January 2013, a total of 2443 *E. coli* isolates from urine, wound, ear, genital tract, anal sac, nasal structure, and soft tissue samples of dogs and cats with presumed naturally-occurring infection in six geographical regions of the United States: West (California), South (North Carolina), Central (Missouri), Midwest (Ohio and Illinois), and Northeast (Alabama, Northeast (Massachusetts) were received from a nationally recognized veterinary diagnostic laboratory. Isolates were reconfirmed to be *E. coli* upon receipt by the Clinical Pharmacology Laboratory (CPL) at Auburn University based on reculture overnight on CHROMagar Orientation (BD Diagnostics, Franklin Lakes, NJ) at 37°C, and then the isolates were harvested and stored in tryptic soy broth containing 30% glycerol at −80°C until studied.

**Susceptibility Testing and Initial ESBL Identification**

Antimicrobial susceptibility testing was performed for all 2443 isolates using 96 well custom microdilution susceptibility plates according to the manufacturer’s protocol (Trek Diagnostic Systems, Inc., Cleveland, OH). Susceptibility testing was performed using 16 antimicrobials representing six antimicrobial classes and classified into 12 antimicrobial categories: penicillins: ampicillin; penicillins + β-lactam inhibitor: amoxicillin-clavulanic acid; anti-pseudomonal + β-lactam inhibitor: ticarcillin-clavulanic acid; non-extended spectrum cephalosporins (1st generation cephalosporins): cephalexin; extended-spectrum cephalosporins (3rd and 4th generation cephalosporins): cefotaxime, ceftazidime, and cefepoxide; cephemycins: cefoxitin; carbapenems: meropenem; tetracyclines: doxycycline; phenicolcs: chloramphenicol; fluoroquinolones: enrofloxacin and ciprofloxacin; aminoglycosides: gentamicin and amikacin; and folate pathway inhibitor: sulfamethoxazole-trimethoprim (Magiorakos et al., 2012; Thungrat et al., 2015). All MIC determinations were performed in triplicates and *E. coli* ATCC 25922 was used for quality control. The results were interpreted according to the guidelines of Clinical Laboratories Standards Institute (CLSI; CLSI, 2013). The MICs were recorded using the Sensititre Vizion system (Trek Diagnostic Systems), and each isolate was categorized in terms of its resistant phenotype as to: susceptible (S), non-multidrug resistance (DR) or MDR. DR was defined as resistance to 1 or 2 antimicrobial classes, and MDR was defined as resistance to three or more antimicrobial classes.

Additionally, all the 2443 *E. coli* isolates were screened for ESBL production using microdilution-based Sensititre (TREK diagnostic systems, Cleveland, Ohio) with ESBL Confirmatory
Aly et al., 2012 described previously (sodium azide resistant E. coli as described previously (Liu et al. ESBL-Producing MDR isolates was determined by use of established PCR assay for ESBL and the level of significance was set at $P < 0.01$; Table 1). Additionally, the occurrence of virulence genes among ESBL-producing MDR E. coli was significantly lower than among non-ESBL isolates with the exception of malX ($P < 0.01$; Table 1).

### Distribution of β-Lactamases and PMQR Genes

The distribution of β-lactamase and PMQR genes among the 68 ESBL-positive MDR E. coli isolates was shown in Table 2. The results showed that $bla_{TEM}$, $bla_{SHV}$, $bla_{CTX-M}$, $bla_{CMY-2}$, and $bla_{OXA-48}$ were detected in 28 (41.2%), 20 (29.4%), 50 (73.5%), 12 (17.6%), and 13 (19.1%) isolates, respectively. 94.1% (64/68) of the isolates harbored two or more β-lactamase genes, and one isolate from dog with severe urinary tract infection co-harbored eight tested genes [$bla_{TEM-5}$, $bla_{SHV-12}$, $bla_{CMY-2}$, $bla_{CTX-M-15}$, $bla_{CTX-M-1}$, $bla_{CTX-M-1-14}$, $bla_{CTX-M-123}$, and $aac(6')-Ib-cr$; Table 2]. For the $bla_{CTX-M}$ positive isolates, CTX-M enzymes were clustered in CTX-M-1 ($n = 35$), CTX-M-9 ($n = 22$), and hybrid β-lactamasers ($n = 27$) clusters. CTX-M-1 and CTX-M-9 double-positive group accounted for 10.3%...
of isolates, and three isolates co-harbored CTX-M-1, CTX-M-9 as well as hybrid β-lactamase. bla\textsubscript{CTX-M−15} \((n = 33)\) was the predominant genotype in bla\textsubscript{CTX-M} positive isolates, and followed by bla\textsubscript{CTX-M−1} \((n = 32)\), bla\textsubscript{CTX-M−123} \((n = 27)\), bla\textsubscript{CTX-M−9} \((n = 19)\), and bla\textsubscript{CTX-M−14} \((n = 19)\). Sequencing of bla\textsubscript{TEM} gene revealed 24 bla\textsubscript{TEM−1}, three bla\textsubscript{TEM−5}, and one bla\textsubscript{TEM−30}, whereas sequencing of bla\textsubscript{SHV} gene revealed 17 bla\textsubscript{SHV−12} and two bla\textsubscript{SHV−3}. All bla\textsubscript{TEM} and bla\textsubscript{SHV} genes were classified as ESBLs with the exception of the bla\textsubscript{TEM−1} gene based on the sequencing. Moreover, 48.5\% \((33/68)\) of investigated isolates harbored aac(6')-Ib-cr, while none of the isolates carried qnr and qepA genes. The vast majority of aac(6')-Ib-cr-producing isolates were positive for bla\textsubscript{CTX-M−15}, bla\textsubscript{CTX-M−1}, and bla\textsubscript{CTX-M−123}, but negative for CTX-M 9 group enzymes despite bla\textsubscript{CTX-M−14} and aac(6')-Ib-cr coexisted in three isolates.

### Conjugation Experiments

We tested whether bla\textsubscript{CTX-M} genes or other β-lactamase genes in 10 selected isolates were transferable by conjugation experiments, and seven out of the 10 ESBL-producing isolates successfully transferred the β-lactamase genes to the recipient E. coli. PCR analysis showed the presence of respective bla\textsubscript{CTX-M} genes and other β-lactamase genes, including two bla\textsubscript{OXA−48}−carrying plasmids from all the transconjugants (Table 3). Meanwhile, PMQR gene aac(6')-Ib-cr was co-transferred with β-lactamase genes. Generally, all donors and their transconjugants were resistant to amoxicillin-clavulanic acid, ampicillin, cephalothin, and ticarcillin-clavulanic acid, and all transconjugants exhibited an increase of at least eight-fold in MICs compared to the recipient, E. coli J53 AZ'. The ciprofloxacin MICs for four transconjugants harboring aac(6')-Ib-cr ranged from 0.06 to 0.125 mg/L, representing an increase of two-fold to four-fold compared with the recipient (Table 3). Additionally, the transconjugants remained susceptible to meropenem, ciprofloxacin, gentamicin, chloramphenicol, and doxycycline, whereas one transconjugant harboring bla\textsubscript{OXA−48} was resistant to sulfamethoxazole-trimethoprim and reduced the susceptibility to meropenem.

### MLST

The MLST investigation revealed that the 68 isolates were assigned to 31 STs, including six new STs (Table 2). Twelve STs were represented by more than two isolates, and other 19 STs contained a single isolate each. ST131 \((n = 10)\), ST648 \((n = 9)\), and ST405 \((n = 9)\) accounted for more than 41\% \((28/68)\) of investigated isolates and 54\% \((27/50)\) of CTX-M-producing isolates, respectively. 74.2\% \((23/31)\) of STs, especially ST131, ST648, and ST405 were positively associated with CTX-M-producing isolates, while other STs, including ST10, ST5232, ST1722, ST2175, ST1800, ST73, ST372, and ST127 seem to have no relationship with CTX-Ms. Vast majority of ST131 and ST648 isolates were positively associated with bla\textsubscript{CTX-M−15} and/or bla\textsubscript{CTX-M−1} as well as bla\textsubscript{CTX-M−123}, whereas 77.8\% of ST405 isolates were negatively associated with bla\textsubscript{CTX-M−1}, bla\textsubscript{CTX-M−15}, and bla\textsubscript{CTX-M−123} genes. Moreover, 55.6\% of ST648 isolates were positively associated with bla\textsubscript{OXA−48}, and 12
TABLE 2 | Occurrence, diversity, and molecular diversity of ESBL-producing MDR isolates.

| Sequence types | Phylogenetic group | Total No. of isolates | β-lactamase genes (No. of isolates) | pAmpC | Carbapenemase | PMQR gene | Resistance profiles |
|---------------|-------------------|-----------------------|------------------------------------|------|--------------|-----------|-------------------|
| Non-ESBL      | ESBL              |                       |                                    |      |              |           |                   |
| ST5174        | F                 | 1                     | CTX-M-1 + CTX-M-15 + CTX-M-123 (1) | CMY-2 (1) |              | aac(6')-Ib-cr (1) | AMC, AMP, OIP, CTX, FOX, CPD, CAZ, CEP, CHL, DOX, ENR, GEN, AMK, TIM, SXT |
| ST1011        | E                 | 1                     | CTX-M-1 + CTX-M-15 (1)             |      |              | aac(6')-Ib-cr (1) | AMC, AMP, OIP, CTX, FOX, CPD, CAZ, CEP, CHL, DOX, ENR, GEN, AMK, TIM, SXT |
| ST10          | A                 | 1                     | TEM-1 (1)                          | CMY-2 (1) |              | aac(6')-Ib-cr (2) | AMC, AMP, OIP, CTX, FOX, CPD, CAZ, CEP, CHL, DOX, ENR, GEN, AMK, TIM, SXT |
| ST167         | A                 | 2                     | CTX-M-1 + CTX-M-15 + CTX-M-123 (2) | CMY-2 (1) |              | aac(6')-Ib-cr (2) | AMC, AMP, OIP, CTX, FOX, CPD, CAZ, CEP, CHL, DOX, ENR, GEN, AMK, TIM, SXT |
| ST5220        | A                 | 1                     | CTX-M-1 + CTX-M-15 + CTX-M-123 (1) |      |              | aac(6')-Ib-cr (1) | AMC, AMP, OIP, CTX, FOX, CPD, CEP, DOX, TIM, SXT |
| ST617         | A                 | 1                     | TEM-5 + CTX-M-15 + CTX-M-123 (1)   |      |              | aac(6')-Ib-cr (1) | AMC, AMP, OIP, CTX, FOX, CPD, CEP, DOX, TIM, SXT |
| ST44          | A                 | 1                     | CTX-M-1 + CTX-M-15 + CTX-M-9 + CTX-M-123 (1) |      |              | aac(6')-Ib-cr (1) | AMC, AMP, OIP, CTX, FOX, CPD, CEP, DOX, ENR, TIM, SXT |
| ST2936        | A                 | 1                     | CTX-M-9 + CTX-M-14 + CTX-M-123 (1) |      |              | aac(6')-Ib-cr (1) | AMC, OIP, CTX, CPD, CAZ, CEP, DOX, ENR, TIM, SXT |
| ST2175        | B1                | 1                     | SHV-12 (1)                         |      |              |           | FOX, CEP, CHL, DOX, GEN, AMK, SXT |
| ST443         | B1                | 1                     | CTX-M-1 + CTX-M-15 (1)             |      |              | aac(6')-Ib-cr (1) | AMC, AMP, OIP, CTX, CPD, CEP, CAZ, CEP, DOX, ENR, TIM, SXT |
| ST162         | B1                | 1                     | TEM-30 + CTX-M-9 + CTX-M-14 + CTX-M-123 (1) | CMY-2 (1) | | OXA-48 (1) | AMC, AMP, OIP, CTX, FOX, CPD, CAZ, MEM, DOX, ENR, TIM |
| ST1800        | B1                | 1                     | CTX-M-9 + CTX-M-14 (1)             | CMY-2 (1) | | OXA-48 (1) | AMC, AMP, OIP, CTX, FOX, CPD, CAZ, MEM, DOX, ENR, TIM |
| ST5231        | C                 | 1                     | CTX-M-1 + CTX-M-15 + CTX-M-123 (1) |      |              | aac(6')-Ib-cr (1) | AMC, AMP, OIP, CTX, FOX, CPD, CAZ, CEP, DOX, ENR, TIM |
| ST5204        | C                 | 1                     | CTX-M-1 + CTX-M-15 + CTX-M-123 (1) |      |              | aac(6')-Ib-cr (1) | AMC, AMP, OIP, CTX, FOX, CPD, CAZ, CEP, DOX, ENR, TIM |
| ST23          | C                 | 2                     | TEM-1 (1)                          | CMY-2 (1) | | aac(6')-Ib-cr (2) | AMC, AMP, OIP, CTX, CPD, CEP, CEP, DOX, ENR, TIM, (FOX) |
| ST5232        | C                 | 1                     | TEM-1 (1)                          | CMY-2 (1) | | aac(6')-Ib-cr (1) | AMC, AMP, OIP, DOX, ENR, TIM |
| ST410         | C                 | 2                     | TEM-1 (2)                          | CMY-2 (1) | | aac(6')-Ib-cr (2) | AMC, AMP, OIP, CTX, CPD, CEP, CEP, DOX, ENR, TIM, SXT |
| ST1088        | C                 | 1                     | TEM-1 (1)                          | OXA-48 (1) | | aac(6')-Ib-cr (2) | AMC, AMP, OIP, CTX, FOX, CPD, CAZ, CEP, MEM, GEN, AMK, TIM, SXT |
| ST1722        | D                 | 1                     | TEM-1 (1)                          | CMY-2 (1) | | aac(6')-Ib-cr (1) | AMC, AMP, CTX, FOX, CPD, CEP, CAZ, CEP, CHL, DOX, TIM, SXT |

(Continued)
| Sequence types | Phylogenetic group | Total No. of isolates | β-lactamase genes (No. of isolates) | PMQR gene | Resistance profiles |
|---------------|-------------------|----------------------|-------------------------------------|------------|---------------------|
|               |                   |                      | Non-ESBL                            | ESBL       | pAmpC              | Carbapenemase     |
|               |                   |                      | CTX-M-1 + CTX-M-15 + CTX-M-123 (1)  |            | aac(6’)-Ib-cr (1)  | AMC, AMP, OIP, CTX, FOX, ODP, CAZ, CEP, CHL, DOX, ENR, TIM, SXT |
| ST68          | D                 | 1                    | CTX-M-1 + CTX-M-15 (1)              |            |                     |                   |
|               |                   |                      | TEM-1 (1)                           | CTX-M-9 + CTX-M-14 + CTX-M-123 (2), SHV-12 (1), CTX-M-1 (1), CTX-M-15 (1),      | aac(6’)-Ib-cr (1) | AMC, AMP, OIP, CTX, FOX, ODP, CAZ, CEP, DOX, TIM, SXT |
| ST69          | D                 | 1                    | CTX-M-1 + CTX-M-15 (1)              | CMY-2 (2)  |                     | AMC, AMP, OIP, CTX, FOX, ODP, CEP, ENR, DOX, TIM, (GEN, AMK, SXT) |
| ST38          | D                 | 3                    | TEM-1 (1)                           | CTX-M-9 + CTX-M-14 (7), CTX-M-1 + CTX-M-15 (2) | OXA-48 (3)  | AMC, AMP, OIP, CTX, FOX, ODP, CAZ, CEP, CHL, DOX, GEN, TIM, (AMK, MEM, SXT) |
| ST405         | D                 | 9                    | TEM-1 (5)                           | CTX-M-9 + CTX-M-14 (2) | OXA-48 (5)  | AMC, AMP, OIP, CTX, FOX, ODP, CAZ, CEP, DOX, ENR, AMK, TIM, SXT, (OHL, MEM, GEN) |
| ST648         | D                 | 9                    | TEM-1 (2)                           | ShV-12 (2), CTX-M-1 + CTX-M-15 + CTX-M-9 (2), CTX-M-1 + CTX-M-15 (3), CTX-M-9 + CTX-M-14 (1), | OXA-48 (2)  | aac(6’)-Ib-cr (7) |
| ST131         | B2                | 10                   | TEM-1 (4)                           | ShV-3 (1), CTX-M-1 + CTX-M-15 + CTX-M-9 (2), ShV-12 (5), CTX-M-1 + CTX-M-14 (2) | OXA-48 (1)  | AMC, AMP, CTX, ODP, CAZ, CEP, CHL, DOX, ENR, GEN, TIM, (CIP, AMK, SKT) |
| ST12          | B2                | 2                    | TEM-1 (1)                           | ShV-12 + CTX-M-14 (1) |                     | AMC, AMP, OIP, CTX, ODP, CAZ, CEP, CHL, DOX, ENR, TIM |
| ST5219        | B2                | 2                    | ShV-12 (1), CTX-M-14 (1)            |             | aac(6’)-Ib-cr (1)  | AMC, CTX, OEP, CHL, DOX, ENR |
| ST961         | B2                | 2                    | ShV-12 (2)                          |             | aac(6’)-Ib-cr (1)  | AMC, CTX, FOX, OEP, CHL, GEN, (OIP, SXT) |
| ST127         | B2                | 2                    | TEM-1 (1)                           | ShV-12 (2)  |                     | AMC, AMP, OIP, CTX, FOX, ODP, CEP, CHL, DOX, ENR, (GEN, AMK, TIM, SXT) |
| ST73          | B2                | 3                    | TEM-1 (2)                           | ShV-12 (1)  |                     | AMC, AMP, OIP, CTX, ODP, CAZ, CEP, DOX, ENR, TIM |
| ST372         | B2                | 3                    | SHV-12 (3)                          |             |                     | AMC, AMP, OIP, CTX, FOX, ODP, CEP, CHL, DOX, ENR, TIM |

AMC, amoxicillin-clavulanic acid; AMR, ampicillin; CTX, cefotaxime; CAZ, cefazolin; FOX, cefoxitin; CFP, cefpodoxime; CEP, cephalothin; MEM, meropenem; DOX, doxycycline; CHL, chloramphenicol; CIP, ciprofloxacin; ENR, enrofloxacin; GEN, gentamicin; AMK, amikacin; TIM, ticarcillin-clavulanic acid; SXT, sulfamethoxazole-trimethoprim.

*a*The antibiotics in parentheses indicated that the antibiotics were variability among the isolates.
pAmpC genes \( \text{bla}_{\text{CMY-2}} \) were distributed in nine STs. Notably, all ST131, ST405, and ST648 isolates expressed resistance to ciprofloxacin and 3rd generation cephalosporins, whereas all ST131 isolates remained susceptible to cefoxitin. A strong correlation was revealed between the virulence gene profiles and STs, and the same STs showed the similar virulence gene profiles. Among the three most common STs, ST405 isolates harbored more virulence genes (mean 4.6), followed by ST131 (mean 4.4), and virulence genes were less abundant in ST648 isolates (mean 3.4). Almost all of the ST131 and ST405 isolates were positive for \( \text{afa} / \text{draBC} \), \( \text{traT} \), and \( \text{mal}X \) genes, ST648 isolates were significantly associated with \( \text{fim}H \), \( \text{mal}X \) and \( \text{traT} \), but negative for \( \text{afa} / \text{draBC} \).

**DISCUSSION**

ESBLs, pAmpC and carbapenemases are mostly responsible for the emerging resistance to the \( \beta \)-lactam antibiotics, especially the 3rd generation cephalosporins and carbapenems in \( E. coli \) (Pitout, 2012). In the present study, we conducted a molecular detection and characterization of the \( \beta \)-lactamase genes in ESBL-producing MDR \( E. coli \) isolates from dogs and cats in the United States over a period of time ranging from August 2009 to January 2013, and also revealed the association between the phylogenetic groups, virulence gene profiles, genetic backbones and \( \beta \)-lactamase types.

The prevalence of 3.8% ESBL-producing \( E. coli \) found in this study is similar to that recorded in a recent study (3%; Shaheen et al., 2011) but higher than the first survey (1%; O’Keefe et al., 2010) among \( E. coli \) from dogs and cats in the United States. Surprisingly, 73.9% (68/92) of the ESBL-producing \( E. coli \) exhibited MDR phenotype, and 75% of MDR isolates were resistant to more than 10 antimicrobial agents tested. Phylogenetic groups D and B2 were the main phylogenetic groups in this study, and it was similar to the phylogenetic subtype distribution of the ESBL-producing isolates from human patients (Hu et al., 2013), which further demonstrated that isolates in phylogenetic groups D and B2 were associated with extraintestinal infections. Among the 68 ESBL-producing MDR isolates, \( \text{bla}_{\text{CTX-M}} \) was prominent and detected in 73.5% (50/68) of isolates, whereas two previous similar surveys carried out in different states in the United States showed that the corresponding prevalence of \( \text{bla}_{\text{CTX-M}} \) were 16.7% and 89.7%, respectively (O’Keefe et al., 2010; Shaheen et al., 2011). It is indicated that the geographical regions, time, resistant phenotype and the history of antimicrobial treatment of the animals can affect the prevalence of \( \text{bla}_{\text{CTX-M}} \) gene. The high prevalence of \( \text{bla}_{\text{CTX-M}} \) strongly suggests a significant role for \( E. coli \) isolates from companion animals as ESBL gene reservoirs, which poses an additional risk to humans. Therefore, monitoring of the spread of \( \text{bla}_{\text{CTX-M}} \) genes in \( E. coli \) isolates in dogs and cats is urgently needed. Although \( \text{bla}_{\text{CTX-M}} \) was still the most frequently encountered gene, the specific genotype of \( \text{bla}_{\text{CTX-M}} \) is undergoing changes, which was supported by available evidence from the occurrence of CTX-M-9 group as well as the occurrence of a novel hybrid \( \beta \)-lactamase gene \( \text{bla}_{\text{CTX-M-123}} \) and \( \text{bla}_{\text{CTX-M-123}} \) was firstly discovered in \( E. coli \) from pig feces in China in 2013 (He et al., 2013), and afterward in human specimen (Hu et al., 2013). It is interesting to note that \( \text{bla}_{\text{CTX-M-15}} \) is also the most widely distributed ESBL gene among human-associated Enterobacteriaceae (Cantón and Coque, 2006). These finding revealed the possibility of cross-transmission between animals and humans. Moreover, several isolates appear only with \( \text{bla}_{\text{TEM-1}} \), \( \text{bla}_{\text{CMY-2}} \), or \( \text{bla}_{\text{OXA-48}} \), suggesting that these isolates perhaps carry other ESBL genes, which will require further studies.

\( \text{bla}_{\text{CMY-2}} \) was the most prevalent pAmpC, and it not only confer resistance to a wide range of extended-spectrum cephalosporins but also are not affected by \( \beta \)-lactamase inhibitors. \( \text{bla}_{\text{CMY-2}} \) was detected in 17.6% of the isolates in our study, and it was significantly lower than the occurrence of \( \text{bla}_{\text{CMY-2}} \) (89%) in \( E. coli \) from companion animals in a previous study in the United States (Shaheen et al., 2011). We supposed that the occurrence of \( \text{bla}_{\text{CMY-2}} \) might be underestimated since only the ESBL-producing MDR isolates were characterized in this study. Meanwhile, our results showed that majority (58.3%) of CMY-2-producing isolates belonged to phylogenetic group D, consistent with a previous study in \( E. coli \) from human in Australia (Sidjabat et al., 2014). This similar distribution of phylogenetic group further certified that \( \text{bla}_{\text{CMY-2}} \) can also be transferred between different bacterial species and between animals and humans (Li et al., 2007; Shaheen et al., 2011). \( \text{bla}_{\text{OXA-48}} \) was initially reported in Klebsiella pneumoniae isolates in Turkey in 2001 (Poirel et al., 2004) and afterward in other Mediterranean countries (Spain, France, Italy, Egypt, and Lebanon Turkey) (Girlich et al., 2014). In 2013, it was firstly discovered in \( E. coli \) from dogs in Germany (Stolle et al., 2013). \( \text{bla}_{\text{OXA-48}} \) can hydrolyze carbapenems and \( \beta \)-lactamase inhibitors but has no activity toward broad-spectrum cephalosporins (Mathers et al., 2013). Our data showed that about 19% of the isolates carried the \( \text{bla}_{\text{OXA-48}} \), and they were mostly associated with meropenem resistance, sequence types ST648, ST405, and ST131 as well as different combinations of \( \beta \)-lactamase genes. To our knowledge, \( \text{bla}_{\text{OXA-48}} \) was firstly reported in the United States in 2012 (Poirel et al., 2012), and the present study is the first report of \( \text{bla}_{\text{OXA-48}} \) in \( E. coli \) from dogs and cats in the United States. Moreover, \( \text{bla}_{\text{OXA-48}} \) can transfer with other \( \beta \)-lactamases and \( \text{aac(6′)-Ib-cr} \). This finding also revealed possibility of the transfer between humans and companion animals appears highly probable through multiple potential pathways although \( \text{bla}_{\text{OXA-48}} \) is still sporadic occurrence in animals.

\( \text{aac(6′)-Ib-cr} \) was the exclusive PMQR gene in this study, and CTX-M-producing isolates (particularly \( \text{bla}_{\text{CTX-M-15}} \) positive isolates) showed significantly higher occurrence of \( \text{aac(6′)-Ib-cr} \) compared to non-CTX-M or non-ESBL isolates (62 vs. 11.1 vs. 10%, \( P < 0.0001 \)). The frequent combination of \( \text{bla}_{\text{CTX-M-15}} \) and \( \text{aac(6′)-Ib-cr} \) in this study further supported the previous studies that coproduction of \( \beta \)-lactamases and PMQR genes could conduce to the dissemination of MDR isolates, and also reflect the fact that genes encoding resistance to \( \beta \)-lactams and quinolones are located on the same plasmid. Although it was not the primary focus of this study, our results coincided
with a previous study (Qin et al., 2013) that ESBL-producing isolates presented a lower occurrence of studied virulence genes compared with non-ESBL isolates (the data from another study in our laboratory) with the exception of malX gene, and CTX-M-producing E. coli harbored fewer virulence genes than non-CTX-M isolates (P<0.0001). A possible reason why individual virulence gene increased among ESBL-producing is that might be a fitness trade-off for the ESBL to survive antibiotics exposure (Qin et al., 2013) and the difference source of E. coli. The exact explanation needs additional study in the future.

A previous review suggested that attention should be paid to the rising of E. coli ST131, ST648, and ST405 isolates as they can play an important role in the worldwide distribution of CTX-M-producing E. coli (Pitout, 2012). It was further confirmed by our results since ST131, ST648, and ST405 accounted for 54% of the CTX-M-producing MDR isolates. ST131 was the predominant clone in this study, and all ST131 isolates remained susceptible to cefoxitin, which has been recently suggested as an alternative carbapenem for the treatment of infections by ESBL-producing E. coli (Gué-Revillet et al., 2014). It is noteworthy that nine ST648 isolates were strongly associated with blaCTX−M−15 (88.9%, 8/9), blaOXA−48 (55.6%, 5/9), and severe clinical signs. The zoonotic potential of ST648-ESBL-producing isolates has been indicated in the isolates from humans, domestic and wild animals in previous studies (Nicolas-Chanoine et al., 2008; Cortes et al., 2010), and two recent studies in Europe further suggested that ST648 clone may represent a novel genotype that combines MDR phenotype, extraintestinal virulence and zoonotic potential in companion animals (Huber et al., 2013; Ewers et al., 2014). Furthermore, ST131, ST648, and ST405 isolates have the similar β-lactam gene combinations and resistance profiles, respectively. While it is alarming that other STs have various β-lactam gene combinations, especially one ST38 isolate, which was associated with the highest frequency of β-lactamases and aac(6′)-Ib-cr, high level cephalosporin resistance (MICs ≥ 32 µg/ml), lowest frequency of virulence genes and severe clinical signs. Nevertheless, constant attention and further investigations for ST648 and ST38 isolates in companion animals are necessary as they are now rapidly and globally disseminated as well as the companion animals are more and more considered an important source of human infections as the physical closeness.

**CONCLUSION**

CTX-M-producing E. coli tend to have less virulent properties compared with the non-CTX-M isolates. CTX-Ms represented by blaCTX−M−1, blaCTX−M−15, and blaCTX−M−123 have spread rapidly. The occurrence of blaCTX−M−123 of ESBLs and blaOXA−48 carbapenemase were particularly striking, being reported here for the first time in E. coli from dogs and cats in the United States. ST131, ST648, and ST405 were the predominant clonal groups among the ESBL-producing E. coli, and all ST131 isolates remained susceptible to the cefoxitin. This information will be useful for assessing...
the epidemiological risk factors and appropriate use of antimicrobials for ESBL-producing *E. coli* infections of dogs and cats.

**AUTHOR CONTRIBUTIONS**

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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**ACKNOWLEDGMENTS**

The authors wish to acknowledge the assistance of laboratory staff in the Clinical Pharmacology Laboratory of Auburn University. This study was supported by the Fundamental Research Funds for the Central Universities (no. 2452016044), National Science Foundation of Shaanxi province (no. 2014JM3071), and partially supported by from Morris Animal Foundation (no. D07-MS006).
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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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