Characterization of ESBL-producing Escherichia coli recovered from companion dogs in Tai’an, China

Song Li¹, Junhe Liu², Yufa Zhou³, Zengmin Miao⁴

¹ College of Basic Medicine, Taishan Medical University, Tai’an, China
² Disease Controlling Center, Veterinary Bureau of Zibo, Zhangdian, China
³ Disease Controlling Center, Veterinary Bureau of Daiyue, Tai’an, China
⁴ College of Life Sciences, Taishan Medical University, Tai’an, China

Abstract

Introduction: Animals are considered to be reservoirs of extended-spectrum beta-lactamase (ESBL)-producing bacteria, but few epidemiological data on ESBL-producing Escherichia coli urinary tract isolates in pet dogs are available in China.

Methodology: This study was conducted to describe the prevalence and characterization of ESBL producers among E. coli urinary tract isolates from pet dogs in Tai’an, China.

Results: A total of 118 E. coli were obtained from urinary samples of 80 companion dogs suffering from acute or chronic cystitis, of which three isolates from different dogs were ESBL producers. One isolate from dog A was of phylogroup A/ST410/CTX-M-15/TEM-1; one from dog B was of phylogroup B1/ST533/CTX-M-15/TEM-1; one from dog C was of phylogroup D/ST648/CTX-M-15. All ESBL producers were resistant to ampicillin, cephalexin, cefalotin, cefpodoxime, ceftiofur, enrofloxacin, marbofloxacin, and trimethoprim/sulfamethoxazole, but were susceptible to imipenem and amoxicillin/clavulanic acid. E. coli of ST533 carrying blCTX-M-15 were first detected in pet dogs in China.

Conclusions: Collectively, the findings could expand our knowledge about the prevalence and characterization of ESBL-producing E. coli urinary tract isolates in pet dogs in China.

Key words: ESBL; Escherichia coli; urinary samples; CTX-M-15; companion dogs.

J Infect Dev Ctries 2017; 11(3):282-286. doi:10.3855/jidc.8138

(Received 19 January 2016 – Accepted 22 March 2016)

Copyright © 2017 Li et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Since the introduction of third-generation cephalosporins in the early 1980s, extended-spectrum beta-lactamase (ESBL)-producing bacteria have rapidly emerged in human and veterinary practices [1]. The main resistance mechanism of these bacteria is the production of ESBLs, but the enzymes can be inhibited by clavulanic acid, sulbactam, and tazobactam [2]. ESBL producers, apart from being resistant to beta-lactam antibiotics, can also be resistant to other classes of antibiotics such as tetracyclines, fluoroquinolones, sulfamethoxazole/trimethoprim, and aminoglycosides [3,4]. There is no doubt that the pan-resistance of ESBL producers limits clinical therapy option and increases medical costs.

ESBL producers were initially detected in human medical practice, but recent investigations have shown that ESBL producers have been found in farm animals and wild animals [5-10]. The increasing number of ESBL-producing isolates found in animals has led to the hypothesis that animals might become infection sources or even reservoirs contributing to the spread of these bacteria [11]. As humans often live in close contact with pets, companion animals could become potential sources of ESBL-producing isolates causing community-acquired infections.

ESBL-producing isolates from companion animals mainly include E. coli and Klebsiella pneumoniae. ESBL-producing E. coli not only are the intestinal pathogen, but also the common causative bacterium for urinary tract infections (UTIs) [11]. To date, numerous investigations about prevalence and characterization of ESBL-producing E. coli from humans and companion animals have been reported [12-17]; however, information about characteristics of ESBL-producing E. coli from pet animals in China is very limited. To fill the literature gap, the present study was designed to describe the prevalence and characterization of ESBL producers among E. coli urinary tract isolates from pet dogs in Tai’an, China.
Methodology

Ethics statement

The study was approved by the ethics committee of Taishan Medical University (permit ECTSMU2011-009).

Bacterial isolates

Between January 2011 and November 2013, urine samples of 80 pet dogs suffering from acute or chronic cystitis were collected by cystocentesis in 6 animal hospitals in Tai’an, China. The collected samples were spread onto blood agar plates and cultured at 37°C for 24 hours. E. coli isolates were identified using traditional biochemical methods and the Vitek2 system (bioMérieux, Hazelwood, USA). The identified isolates were stored at -20°C in cryoprotective media prior to use.

Phenotypic ESBL detection and antimicrobial susceptibility testing

According to the manufacturer's protocols, Etest ESBL strips (bioMérieux, Marcy l’Étoile, France) were used to determine ESBL production of E. coli isolates. According to the Clinical and Laboratory Standards Institute guidelines [18], agar dilution method was used to test susceptibility of ESBL-producing E. coli isolates against 12 antimicrobial agents. The tested drugs included ampicillin, cephalaxin, cefalotin, cepodoxime, ceftriaxone, meropenem, tetracycline, amoxicillin/clavulanic acid, trimethoprim/sulfamethoxazole, and amikacin (Tianhe, Hangzhou, China). E. coli ATCC 25922 was used as a quality control strain.

If ESBL-producing E. coli isolates obtained from the same individual companion dog showed the same drug-resistant phenotype, ESBL gene, and multilocus sequence type (ST), these isolates were considered to be the same strain and only one was included in this study. An E. coli isolate was considered to be multidrug-resistant (MDR) when it exhibited resistance to antimicrobials of at least three different classes [19].

Detection of beta-lactamase genes

Polymerase chain reaction (PCR) was used in this study to amplify beta-lactamase resistance genes (blaCTX-M, blaTEM, and blaSHV) for all ESBL-producing isolates, and the corresponding primers and reaction conditions were used as previously described [16]. The amplified products were either directly sequenced from both ends or cloned in pMD18-T and then sequenced. The deduced amino acid sequences were aligned using Lasergene software (DNASTAR, Madison, USA), and compared with sequences available at GenBank (http://www.ncbi.nlm.nih.gov/GenBank/index.html) to determine ESBL genotype. Mutations were also analyzed with reference to the Lahey Clinic website (http://www.lahey.org/studies/).

Determination of E. coli phylogroups in ESBL producers

E. coli has four main phylogroups (A, B1, B2, and D), among which groups A and B1 typically contain commensal isolates and strains of groups B2 and D are more likely to carry pathogenicity-associated genes [20,21]. The analysis of phylogenetic groups was carried out using multiplex PCR, according to the method described previously [22].

Multilocus sequence typing of ESBL-producing E. coli

According to the previous reference [23], the internal fragments of seven housekeeping genes (adk, fumC, gyrB, icd, mdh, purA, and recA) were sequenced. The alleles and multilocus ST were assigned based on the E. coli MLST website (http://mlst.ucc.ie/mlst/dbs/Ecoli).

Results

A total of 118 E. coli isolates were obtained from urinary samples of 80 pet dogs suffering from acute or chronic cystitis in 6 animal hospitals in Tai’an city between January 2011 and November 2013. In total, 3 ESBL-producing E. coli isolates were isolated from urinary samples of 3 pet dogs (A, B, and C): 1 isolate from dog A (a male Pekingese), 1 from dog B (a female Pekingese), and 1 from dog C (a male Golden Retriever) (Table 1).

A total of 3 ESBL–producing E. coli isolates in this study were all MDR and were all susceptible to amoxicillin/clavulanic acid and imipenem. In addition,

Table 1. Characterization of ESBL-producing E. coli in this study.

| Origin | No. of isolates | MLST | β-lactamase identified | Phylogroup |
|--------|----------------|------|------------------------|------------|
| Dog A  | 1              | ST410| CTX-M-15, TEM-1        | A          |
| Dog B  | 1              | ST533| CTX-M-15, TEM-1        | B1         |
| Dog C  | 1              | ST648| CTX-M-15               | D          |

ESBL: extended-spectrum beta-lactamase; MLST: multilocus sequence typing.
2 isolates from dogs B and C were resistant to amikacin (Table 2).

Among 3 ESBL-producing E. coli isolates, 1 strain from dog A belonged to phylogroup A, carried blaCTX-M-15 and blatem-1 genes, and was of ST410; 1 strain from dog C was of ST648, carried blaCTX-M-15, and belonged to phylogroup D; and 1 isolate from dog B belonged to phylogroup B1, contained blaCTX-M-15 and blatem-1 genes, and was of ST533 (Table 1).

### Discussion

All ESBL-producing E. coli isolates in this study were resistant to ampicillin, cephalexin, cefalotin, cefpodoxime, cefotiofur, enrofloxacin, marbofloxacin, tetracycline, and trimethoprim/sulfamethoxazole. The result may be related to the fact that plasmids containing blaCTX-M often carry resistance genes, such as fluoroquinolones and aminoglycosides [4,24]. However, ESBL-producing E. coli isolates were all susceptible for amoxicillin/clavulanic acid and imipenem. In addition, only one ESBL-producing E. coli isolate from dog A was susceptible to amikacin.

E. coli of ST410 carrying the blaCTX-M-15 gene has been detected in dog urinary samples and human samples in China and other countries [16,25-27]. E. coli of ST648 carrying blaCTX-M-15 gene has been frequently found in clinical ESBL-producing E. coli isolates from humans and animals worldwide [15,16,24,28], and therefore the ESBL-producing E. coli of ST648 is regarded as the potential extended-host spectrum genotype. ESBL-producing E. coli of ST533 is sparse and was only detected twice in humans with UTIs in Brazil and Germany [29], once in manure samples of gulls in France [30], and once in urinary samples of pet dogs in Switzerland [16]. To our best knowledge, E. coli of ST533 carrying blaCTX-M-15 was detected in companion dog for the first time in China.

In this study, all three ESBL-producing E. coli isolated from urinary samples of companion dogs carried the blaCTX-M-15 gene, and two of three ESBL-producing E. coli carried the blatem-1 gene. blaCTX-M-15+TEM was the dominant bla gene type, which is consistent with the results of other studies detecting these genes in ESBL producers from companion animals in China and other counties [11,16,31]. CTX-M-15-producing E. coli of ST131 was not found in the present study, which is regarded as an emerging human pandemic clone [32]. However, ESBL-producing E. coli has been detected in urinary samples of dogs in Europe [12]. Additionally, no blasIV gene was found in this study, which is in agreement with the results of other studies about ESBL-producing E. coli from dogs in China [33,34]. However, in the United States, blasIV-12 was detected from urinary samples of companion animals [13].

### Conclusions

In summary, the limitation of this study was the relatively small number of ESBL-producing E. coli from pet dogs. The findings of this study, however, could improve our knowledge about the prevalence and characterization of ESBL-producing E. coli urinary tract isolates in pet dogs in China.

### Acknowledgements

This study was supported by the National Natural Science Foundation of China (81501357).

---

**Table 2. Antibiotic susceptibility of ESBL-producing E. coli in this study.**

| Antimicrobials             | Dog A          | MIC (µg/mL) | Interpretation | Dog B          | MIC (µg/mL) | Interpretation | Dog C          | MIC (µg/mL) | Interpretation |
|----------------------------|----------------|-------------|----------------|----------------|-------------|----------------|----------------|-------------|----------------|
| Ampicillin                 | R              | ≥ 32        | S              | R              | ≥ 32        | R              | ≥ 32           |             |                |
| Cefalexin                  | R              | ≥ 64        | S              | R              | ≥ 64        | R              | ≥ 64           |             |                |
| Cefpodoxime                | R              | ≥ 8         | S              | R              | ≥ 8         | R              | ≥ 8            |             |                |
| Cefotiofur                 | R              | ≥ 8         | S              | R              | ≥ 8         | R              | ≥ 8            |             |                |
| Cefpirome                  | R              | 16          | S              | R              | 32          | R              | 16             |             |                |
| Imipenem                   | S              | ≤ 1         | S              | S              | ≤ 1         | S              | ≤ 1            |             |                |
| Amikacin                   | S              | ≤ 2         | R              | 16             | R           | 16             |                |             |                |
| Enrofloxacin               | R              | ≥ 4         | R              | ≥ 4            | R           | ≥ 4            |                |             |                |
| Marbofloxacin              | R              | ≥ 4         | R              | ≥ 4            | R           | ≥ 4            |                |             |                |
| Tetracycline               | R              | ≥ 16        | R              | ≥ 16           | R           | ≥ 16           |                |             |                |
| amoxicillin/clavulanic acid| S              | ≤ 8         | S              | ≤ 8            | S           | ≤ 8            |                |             |                |
| trimethoprim/sulfamethoxazole| R            | ≥ 4/76      | R              | ≥ 4/76         | R           | ≥ 4/76         |                |             |                |

ESBL: extended-spectrum beta-lactamase; MIC: minimum inhibitory concentration; S: susceptible; R: resistant (intermediate results were considered resistant).
References
1. Kliebe C, Nies BA, Meyer JF, Tolxdorf-Neutzung RM, Wiedemann B (1985) Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. Antimicrob Agents Chemother 28: 302-307.

2. Bush K, Jacoby GA (2010) Updated functional classification of β-lactamases. Antimicrob Agents Chemother 54: 969-976.

3. Winokur PL, Canton R, Casellas JM, Legakis N (2001) Variations in the prevalence of strains expressing an extended-spectrum β-lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific region. Clin Infect Dis 32: 94-103.

4. Coque TM, Baquero F, Canton R (2008) Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Euro Surveill 13: 5437-5453.

5. Paterson DL, Ko WC, Von Gottberg A, Mohapatra S, Casellas JM, Goossens H, Mulazimoglu L, Trenholme G, Klugman KP, Bonomo RA, Rice LB, Wagener MM, McCormack JG, Yu VL (2004) International prospective study of Klebsiella pneumoniae bacteremia: implications of extended-spectrum β-lactamase production in nosocomial Infections. Ann Intern Med 140: 26-32.

6. Pitout JD, Nordmann P, Laupland KB, Poirel L (2005) Emergence of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) in the community. J Antimicrob Chemother 56: 52-59.

7. Blane V, Mesa R, Saco M, Lavilla S, Prats G, Miro E, Navarro F, Cortés P, Llagostera M (2006) ESBL- and plasmidic class C β-lactamase-producing E. coli strains isolated from poultry, pig and rabbit farms. Vet Microbiol 118: 299-304.

8. Costa D, Poeta P, Saenz V, Vinue L, Rojo-Bezares B, Jouini A, Zarazaga M, Rodrigues J, Torres C (2006) Detection of Escherichia coli harbouring extended-spectrum β-lactamases of the CTX-M, TEM and SHV classes in faecal samples of wild animals in Portugal. J Antimicrob Chemother 58: 1311-1312.

9. Watson E, Jeckel S, Snow L, Stubbs R, Teale C, Wearing H, Horton R, Toszeghy M, Tearne O, Ellis-Iversen J, Coldham N (2012) Epidemiology of extended spectrum β-lactamase E. coli (CTX-M-15) on a commercial dairy farm. Vet Microbiol 154: 339-346.

10. Hordijk J, Schoormans A, Kwakernaak M, Duin B, Broens E, Dierikx C, Mevius DJ, Wageman JA (2013) High prevalence of fecal carriage of extended spectrum β-lactamase/AmpC-producing Enterobacteriaceae in cats and dogs. Front Microbiol 4: 242.

11. Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH (2012) Extended-spectrum β-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: a global perspective. Clin Microbiol Infect 18: 646-655.

12. Ewers C, Grobbl M, Stamm I, Kopp PA, Diehl I, Semmler T, Fruth A, Beutlich J, Guerra B, Wieler LH, Guenther S (2010) Emergence of human pandemic O25:H4-ST131 CTX-M-15 extended-spectrum-b lactamase-producing Escherichia coli among companion animals. J Antimicrob Chemother 65: 651-660.

13. O’Keefe A, Hutton TA, Schifferli DM, Rankin SC (2010) First detection of CTX-M and SHV extended-spectrum beta-lactamases in Escherichia coli urinary tract isolates from dogs and cats in the United States. Antimicrob Agents Chemother 54: 3489-3492.

14. Bourjilat F, Bouchrif B, Dersi N, Claude JD, Amarouch H, Timinouni M (2011) Emergence of extended-spectrum beta-lactamases-producing Escherichia coli in community-acquired urinary infections in Casablanca, Morocco. J Infect Dev Ctries 5: 850-855. doi:10.3855/jidc.1490.

15. Dierikx CM, van Duijkeren E, Schoormans AH, van Essen-Zandbergen A, Veldman K, Kant A, Huijdens XW, van der Zwaluw K, Wagenaar JA, Mevius DJ (2012) Occurrence and characteristics of extended-spectrum β-lactamase- and AmpC-producing clinical isolates derived from companion animals and horses. J Antimicrob Chemother 67: 1368-1374.

16. Huber H, Zweifel C, Wittenbrink MM, Stephan R (2013) ESBL-producing uropathogenic Escherichia coli isolated from dogs and cats in Switzerland. Vet Microbiol 162: 992-996.

17. Nam EH, Ko S, Chae JS, Hwang CY (2013) Characterization and zoonotic potential of uropathogenic Escherichia coli isolated from dogs. J Microbiol Biotechnol 23: 422-429.

18. Clinical and Laboratory Standards Institute (CLSI) (2015) Performance standards for Antimicrobial Susceptibility Testing. 25th Informational Supplement. CLSI document M100-S25. Wayne, USA: CLSI.

19. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18: 268-281.

20. Pupo GM, Karaoilis DKR, Lan R, Reeves PR (1997) Evolutionary relationships among pathogenic and nonpathogenic Escherichia coli strains inferred from multilocus enzyme electrophoresis and mdh sequence studies. Infect Immun 65: 2685-2692.

21. Picard B, Garcia JS, Gouriou S, Duriez P, Brahami N, Bingen E, Elion J, Denamur E (1999) The link between phylogeny and virulence in Escherichia coli extraintestinal infection. Infect Immun 67: 546-553.

22. Clermont O, Bonacorsi S, Bingen E (2000) Rapid and simple determination of the Escherichia coli phylogenetic group. Appl Environ Microbiol 66: 4555-4558.

23. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MCJ, Ochman H, Achtmann M (2006) Sex and virulence in Escherichia coli: an evolutionary perspective. Mol Microbiol 60: 1136-1151.

24. Wieler LH, Ewers C, Guenther S, Walther B, Lübke-Becker A (2011) Methicillin-resistant staphylococci (MRS) and extended-spectrum b lactamases (ESBL)-producing Enterobacteriaceae in companion animals: nosocomial infections as one reason for the rising prevalence of these potential zoonotic pathogens in clinical samples. Int J Med Microbiol 301: 635-641.

25. Schink AK, Kadlec K, Schwarz S (2011) Analysis of blACTX-M- carrying plasmids from Escherichia coli isolates collected in the BFT-GermVet study. Appl Environ Microbiol 77: 7142-7146.

26. Sidjabat HE, Paterson DL, Adams-Haduch JM, Ewan L, Pascullle AW, Muto CA, Tian GB, Doi Y (2009) Molecular epidemiology of CTX-M-producing Escherichia coli isolates at a tertiary medical center in western Pennsylvania. Antimicrob Agents Chemother 53: 4733-4739.

27. Zhang J, Zheng B, Zhao L, Wei QZ, Ji JR, Li LJ, Xiao YH (2014) Nationwide high prevalence of CTX-M and an increase of CTX-M-55 in Escherichia coli isolated from patients with community-onset infections in Chinese county hospitals. BMC Infect Dis 14: 659.

28. van der Bij AK, Peirano G, Pitoondo-Silva A, Pitout JD (2012) The presence of genes encoding for different virulence factors...
in clonally related *Escherichia coli* that produce CTX-Ms. Diagn Microbiol Infect Dis 72: 297-302.

29. Minarini LA, Camargo IL, Pitondo-Silva A, Darini AL (2007) Multilocus sequence typing of uropathogenic ESBL-producing *Escherichia coli* isolated in a Brazilian community. Curr Microbiol 55: 524-529.

30. Bonnedahl J, Drobin M, Gauthier-Clerc M, Hernandez J, Granholm S, Kayser Y, Melhus A, Kahlmeter G, Waldenstrom J, Johansson A, Olsen B (2009) Dissemination of *Escherichia coli* with CTX-M type ESBL between humans and yellow-legged gulls in the south of France. PLoS ONE 4: e5958.

31. Sun Y, Zeng Z, Chen S, Ma J, He L, Liu Y, Deng Y, Lei T, Zhao J, Liu JH (2010) High prevalence of *blaCTX-M* extended-spectrum beta-lactamase genes in *Escherichia coli* isolates from pets and emergence of CTX-M-64 in China. Clin Microbiol Infect 16: 1475-1481.

32. Johnson JR, Johnston B, Clabots C, Kuskowski MA, Castanheira M (2010) *Escherichia coli* sequence type ST131 as the major cause of serious multidrug-resistant *E. coli* infections in the United States. Clin Infect Dis 51: 286-294.

33. Cui YM, Song XY, Wang LF, Liu BG, Tian YK, Hu GZ (2015) Detection of resistance of dog-derived *Escherichia coli* isolates to tetracyclines. Acta Agr Jiangxi 27: 104-107.

34. Zhao XS, Sun Y, Ji X, Liu J, Zhu LW, Tong PP, Feng SZ (2014) Antibiotic resistance of *Escherichia coli* isolated from dogs. Chin J Zoonoses 30: 268-272.

**Corresponding author**
Zengmin Miao  
College of Life Sciences, Taishan Medical University  
Changcheng Road 619, Tai'an 271000, China,  
Phone: +08605386236603  
Fax: +08605386236603  
Email: zengminmiao@126.com

**Conflict of interests:** No conflict of interests is declared.