JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Brief Original Article

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

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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 *bla*$_{\text{CTX-M-15}}$ 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)

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 β-lactam antibiotics, can also be resistant to other classes of antibiotics such as tetracyclines, fluoroquinolones, sulfmethoxazole/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'Etoile, 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, cephalexin, cefalotin, cefpodoxime, ceftiofur, enrofloxacin, marbofloxacin, imipenem, 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 (bla_{CTX-M}, bla_{TEM}, and bla_{SHV}) 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.

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

Antimicrobials	Dog A		Dog B		Dog C	
	Interpretation	MIC (μg/mL)	Interpretation	MIC (μg/mL)	Interpretation	MIC (μg/mL)
Ampicillin	R	≥ 32	R	≥ 32	R	≥ 32
Cefalexin	R	≥ 64	R	≥ 64	R	≥ 64
Cefpodoxime	R	≥ 8	R	≥ 8	R	≥ 8
Ceftiofur	R	≥ 8	R	≥ 8	R	≥ 8
Cefpirome	R	16	R	32	R	16
Imipenem	S	≤ 1	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).

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 $bla_{\text{CTX-M-15}}$ and $bla_{\text{TEM-1}}$ genes, and was of ST410; 1 strain from dog C was of ST648, carried $bla_{\text{CTX-M-15}}$, and belonged to phylogroup D; and 1 isolate from dog B belonged to phylogroup B1, contained $bla_{\text{CTX-M-15}}$ and $bla_{\text{TEM-1}}$ genes, and was of ST533 (Table 1).

Discussion

All ESBL-producing *E. coli* isolates in this study were resistant to ampicillin, cephalexin, cefalotin, cefpodoxime, ceftiofur, enrofloxacin, marbofloxacin, tetracycline, and trimethoprim/sulfamethoxazole. The result may be related to the fact that plasmids containing $bla_{\text{CTX-M}}$ 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 $bla_{\text{CTX-M-15}}$ gene has been detected in dog urinary samples and human samples in China and other countries [16,25-27]. *E. coli* of ST648 carrying $bla_{\text{CTX-M-15}}$ 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 $bla_{\text{CTX-M-15}}$ 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 $bla_{\text{CTX-M-15}}$ gene, and two of three ESBL-producing *E. coli* carried the $bla_{\text{TEM-1}}$ gene. $bla_{\text{CTX-M+TEM}}$ 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 bla_{SHV} 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, $bla_{\text{SHV-12}}$ 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).

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Conflict of interests: No conflict of interests is declared.