Polish Journal of Microbiology
2015, Vol. 64, No 3, 285–288

SHORT COMMUNICATION

Characterization of Extended-Spectrum-β-Lactamases Produced by *Escherichia coli* Strains Isolated from Dogs in Poland

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Submitted 25 March 2015, accepted 8 April 2015

Abstract

Escherichia coli is a common cause of infections in companion animals. In recent years the increasing prevalence of resistance to β-lactams, including extended-spectrum cephalosporins, antimicrobials frequently used in small animal veterinary practice, was observed in canine isolates of *E. coli*. The aim of this study was to detect and to characterize extended-spectrum β-lactamases (ESBLs) produced by *E. coli* isolated from diseased dogs in Poland. Four isolates out of 119 studied (3.4%) were ESBL-positive. They harbored the $bla_{SHV\text{-}12}$, $bla_{CTX\text{-}M\text{-}15}$, and $bla_{TEM\text{-}116}$ genes. This study provides the first report of the occurrence of ESBL-producing *E. coli* in dogs in Poland.

Key words: *Escherichia coli*, extended-spectrum β-lactamases, dog infections, multidrug resistance

Escherichia coli is an important opportunistic pathogen, causing in dogs mainly extraintestinal infections including those of urinary, respiratory and reproductive tracts. The antimicrobial resistance of *E. coli* occurring in companion animals, especially the multidrug resistance, becomes an emerging problem in veterinary medicine. The increasing percentage of multidrug resistant (MDR) *E. coli* isolation from dogs and cats in Poland, between 2007 and 2013, has been reported by Rzewuska *et al*. (2015). The increase in the prevalence of resistance to β-lactams, such as aminopenicillins and extended-spectrum cephalosporins, was also observed in canine *E. coli* isolates. The β-lactam resistance in *Enterobacteriaceae* is associated mainly with production of enzymes hydrolyzing these antibiotics, among which the extended-spectrum β-lactamases (ESBLs), plasmidic AmpC β-lactamases and carbapenemases are the most important resistance mechanisms (Rubin and Pitout, 2014). ESBLs mediate resistance to penicillins, cephalosporins and monobactams, but they are sensitive to β-lactam inhibitors. The presence of ESBL-producing *E. coli* in clinical specimens from dogs has been reported previously in some countries, such as the United States (O'Keefe *et al*., 2010; Shaheen *et al*., 2011), the Netherlands (Dierikx *et al*., 2012; Hordijk *et al*., 2013), Germany (Schmiedel *et al*., 2014), Italy (Carattoli *et al*., 2005) and Korea (So *et al*., 2012). However, detailed information about properties of ESBLs occurring in canine *E. coli* and their geographic distribution are still limited. To our knowledge, there are no published data regarding the occurrence of ESBLs in canine *E. coli* in Poland.

The aim of the study was to detect and to characterize ESBLs in *E. coli* isolated from diseased dogs in Poland.

E. coli isolates (n = 119) investigated in this study were obtained from clinical samples collected from diseased dogs. The isolates were identified using standard microbiological diagnostic techniques. Antimicrobial susceptibility was determined by the disk diffusion method, as described previously (Rzewuska *et al*., 2015). *E. coli* ATCC 25922 was used as a quality control.

The phenotypic test using ceftazidime and ceftazidime/clavulanic acid disks (Becton Dickinson) was performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2013) to detect ESBLs production.

The presence of bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M\text{-}1\ \text{group}}$ and $bla_{CTX\text{-}M\text{-}9\ \text{group}}$ genes was studied by PCR to determine the genotype of ESBL-positive isolates. In addition,

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Table I
Primers used to detect genes encoding β-lactamases in the study

Target gene	Primer sequence (5'-3')	Amplicon size (bp)	Literature
bla_{TEM}	F-ATTCTTGAAGACGAAAGGGC	1150	Briñas *et al.*, 2005
	R-ACGCTCAGTGGAACGAAAAC		
bla_{SHV}	F-CACTCAAGGATGTATTGTG	885	Briñas *et al.*, 2005
	R-TTAGCGTTGCCAGTGCTCG		
$bla_{CTX\text{-}M\text{-}1\ group}$	F-GTTACAATGTGTGAGAAGCAG	1049	Costa *et al.*, 2008
	R-CCGTTTCCGCTATTACAAAC		
$bla_{CTX\text{-}M\text{-}9\ group}$	F-GTGACAAAGAGAGTGCAACGG	857	Coque *et al.*, 2002
	R ATGATTCTCGCCGCTGAAGCC		
$bla_{CMY\text{-}2}$	F-GATTCCTTGGACTCTTCAG	1807	Briñas *et al.*, 2005
	R-TAAAACCAGGTTCCCAGATAGC		

those isolates were screened for the $bla_{CMY\text{-}2}$ gene, as the activity of β-lactamase CMY-2 could mask the ESBL-positive phenotype (Thomson, 2010). The PCR assays were performed using primers (Genomed, Poland) and reaction conditions described previously (Table I). DNA was isolated using Genomic Mini kit (A&A Biotechnology, Poland) according to the manufacturer's recommendations. In order to identify the type of genes detected, the obtained amplicons were purified with the GeneJET™ PCR Purification Kit (Thermo-Scientific) according to the manufacturer's recommendations, and sequenced using the same primers and a 3730 *xl* DNA Analyzer (Applied Biosystems, USA). Sequencing files were evaluated using the Chromas Lite version 2.33 program (Technelysium Pty Ltd., Australia). Subsequently, the nucleotide sequences were compared to the sequences in the GenBank database using BLAST (http://blast.ncbi.nlm.nih.gov). Additionally, the bla_{TEM} nucleotide sequences were translated into protein sequences, and then aligned with the reference sequence of TEM-1 β-lactamase (GenBank Accession Number J01749) by MEGA version 5.0. On the basis of the amino acid substitutions found and the TEM mutation table (http://www.lahey.org/Studies/temtable.asp), the type of TEM β-lactamase was determined for each bla_{TEM} gene detected.

ESBL-producing *E. coli* was detected among the studied isolates, and this is the first report on the presence of this bacterium in dogs in Poland. The ESBL-positive phenotype was found in four *E. coli* isolates from extraintestinal infections in dogs. Characteristics of these isolates are presented in Table II. Genes of three different ESBLs were detected and identified, as $bla_{SHV\text{-}12}$, $bla_{CTX\text{-}M\text{-}15}$, and $bla_{TEM\text{-}116}$. The fourth gene whose presence was assayed, $bla_{CMY\text{-}2}$ encoding a plasmidic class C β-lactamase CMY-2, was not found in any of those isolates.

In the present study all ESBL-producing *E. coli* isolates were classified as MDR bacteria, showing resistance to at least three antimicrobial classes (Table III). Multidrug resistance has been also observed in ESBL-positive *E. coli* of various origin in other studies (Schmiedel *et al.*, 2014; Shaheen *et al.*, 2011).

The occurrence of ESBL-producing *E. coli* in dogs, ranging from 1% to 33.3%, has been reported previously (Dierikx *et al.*, 2012; Ewers *et al.*, 2010; Hordijk *et al.*, 2013; Huber *et al.*, 2013; O'Keefe *et al.*, 2010; Schmiedel *et al.*, 2014; Shaheen *et al.*, 2011; So *et al.*, 2012). Ewers *et al.* (2010) reported that ESBL-producing *E. coli* was isolated from 10.7% of clinical samples collected from dogs. The high prevalence (33.3%) of these bacteria isolated from rectal swabs of hospitalized dogs in Korea was reported by So *et al.* (2012). In our study, only 3.4% (4/119 isolates) of studied *E. coli* isolates were ESBL-positive. These findings correspond with the observations of Shaheen *et al.* (2011) in the

Table II
Characteristics of ESBLs produced by *E. coli* isolates obtained from dogs

Strain designation	Clinical sample	ESBL type		
		bla_{SHV}	bla_{TEM}	bla_{CTX}
1062/09/D	pharyngeal swab	–	TEM-116	CTX-M-15
1370/06/D	ear canal swab	–	TEM-116	CTX-M-15
1945/06/D	nasal swab	SHV-12	TEM-116	–
2017/11/D	soft tissue (liver)	–	TEM-116	CTX-M-15

Table III
Antimicrobial susceptibility of ESBL-producing *E. coli* strains isolated from dogs

Antimicrobial	1062/09/D	1370/06/D	1945/06/D	2017/11/D
Amoxicillin	R	R	R	R
Amoxicillin/clavulanic acid	R	R	R	R
Cefuroxime	R	R	R	R
Cefotaxime	R	R	R	R
Cefovecin	R	R	R	R
Ciprofloxacin	R	R	S	R
Enrofloxacin	R	R	S	R
Marbofloxacin	R	R	S	R
Tetracycline	R	R	R	R
Gentamicin	S	S	R	R
Nitrofurantoin	R	R	R	R
Colistin	S	S	R	R
Florfenicol	S	S	R	R
Trimethoprim/Sulfamethoxazole	S	R	R	R

R – resistant, S – susceptible

United States and Huber *et al*. (2013) in Switzerland, where the frequency of ESBL-producing *E. coli* isolation from dogs, mainly from urinary tract infections, was 3% and 3.3%, respectively.

Three different types of ESBLs were found in the studied *E. coli* isolates (Table II). The β-lactamase CTX-M-15, detected in three isolates, belongs to the CTX-M-1 group and represents the most frequently reported ESBL type in *E. coli* isolates of canine and feline origin (Ewers *et al*., 2010; Huber *et al*., 2013; O'Keefe *et al*., 2010; Schmiedel *et al*., 2014; Shaheen *et al*., 2011). The other ESBL, SHV-12, detected in one of the studied isolates, has rarely been found in *E. coli* isolated from dogs (Carattoli *et al*., 2005; Ewers *et al*., 2010; O'Keefe *et al*., 2010). Furthermore, in all ESBL-positive isolates the gene encoding the TEM-116 β-lactamase was detected. This enzyme is TEM-1 derivative with ESBL activity, and occurs in various species of *Enterobacteriaceae* isolated from humans (Dhara *et al*., 2013). This is only the second report of TEM-116 β-lactamase in *E. coli* of canine origin, the first being that of Ewers *et al*. (2010).

In this study ESBL-producing *E. coli* strains were isolated from diseased dogs with extraintestinal infections. However, they have also been detected in faecal samples of healthy dogs and cats (Belas *et al*., 2014; Hordijk *et al*., 2013), and it seems that companion animals could be asymptomatic carriers of these bacteria.

The β-lactams are antimicrobial drugs commonly used in small animal veterinary practice (Murphy *et al*., 2012). The β-lactamases, which mediate the β-lactam resistance, are most often encoding by genes grouped in cassettes and located on mobile genetic elements, such as plasmids and transposons, so they may be extensively transmitted between different bacteria. Therefore inappropriate usage of β-lactams may contribute to the development of broad-spectrum resistance and to the dissemination of multiresistant strains among humans and animals. Our study showed that dogs in Poland can be a potential reservoir of ESBL-positive *E. coli*, though the prevalence of these bacteria in clinical specimen was relatively low. The results suggest that the ESBL production is probably not a main mechanism of resistance to β-lactams in the studied *E. coli* population. However, the further investigation should explain a role of other resistance mechanisms in *E. coli* of canine origin.

Acknowledgements

The authors thank Barbara Chojnacka and Alicja Grzechnik for excellent technical assistance.

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