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

MAGDALENA RZEWUSKA1∗, ILONA STEFAŃSKA3, MAGDALENA KIZERWETTER-ŚWIDA1, DOROTA CHROBAK-CHMIEL3, PAULINA SZCZYGIELSKA1, MONIKA LEŚNIAK2 and MARIAN BINEK1

1 Department of Preclinical Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland
2 Department of Fermentation Technology, Institute of Agricultural and Food Biotechnology, Warsaw, Poland
3 Department of Regenerative Medicine, Military Institute of Hygiene and Epidemiology, Warsaw, Poland

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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 blaSHV-12 and blaTEM-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

∗ Corresponding author: M. Rzewuska, Department of Preclinical Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland; e-mail: magdalena_rzewuska@sggw.pl
those isolates were screened for the \textit{bla}_{\text{CMY-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 \textit{bla}_{\text{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 \textit{bla}_{\text{TEM}} gene detected.

ESBL-producing \textit{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 \textit{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 \textit{bla}_{\text{SHV-12}} \textit{bla}_{\text{CTX-M-15}} and \textit{bla}_{\text{TEM-116}}. The fourth gene whose presence was assayed, \textit{bla}_{\text{CMY-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 \textit{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 \textit{E. coli} of various origin in other studies (Schmiedel et al., 2014; Shaheen et al., 2011).

The occurrence of ESBL-producing \textit{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 \textit{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 \textit{E. coli} isolates were ESBL-positive. These findings correspond with the observations of Shaheen et al. (2011) in the
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.

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**Literature**


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