Introduction

ESBLs enzymes confer resistance to extended-spectrum cephalosporins (ESCs) and monobactams and often express a resistance to non-β-lactam antimicrobials such as fluoroquinolones leaving only limited therapeutic options (Pitout et al., 2008). *Escherichia coli* strains colonize the digestive tract both in animals, and in humans. Resistance to ESC is one of the characteristics commonly seen in *E. coli* strains, representing an issue both for human and animal health (Ljungquist et al., 2016). The change of faecal microbiota in dogs, with increased resistance and carriage of ESBL producing *E. coli* isolates is more prevalent with most dogs being intermittently shedders and positive carriers for various ESBL genes (Baede et al., 2015). Several studies have shown that the most common type of ESBL enzyme isolated from dog faecal microbiota was CTX-M-15 a predominant subtype both in humans and in...
animals, including in livestock and wild animals (Brolund et al., 2016; Ewers et al., 2012; Guenther et al., 2011). Bacterial resistance to antibiotics, seen in Enterobacteriaceae strains isolated from dogs, may represent a potential risk for public health due to potential of being a reservoir of resistant bacteria for people with whom animals may come in direct contact (physical injuries, petting licking) or indirect contact (contamination of the household environment) (Guardabassi et al., 2004). With the exception of a study looking at antibiotic resistance of E. coli strains isolated from chicken (Măciucă et al., 2015) characterization of ESC-resistance resistance in animals has received less attention in Romania.

The aim of this work was to characterise antimicrobial resistance among E. coli isolated from dog faeces from two shelters in the North-East of Romania, with an emphasis on extended spectrum cephalosporin (ESC) resistance. The main focus was to determine the prevalence of β-lactamase (TEM, SHV, OXA), extended-spectrum β-lactamase (ESBL) and genes encoding plasmid mediated resistance to quinolones (PMQR) in ESC-resistant E. coli isolated from healthy dogs.

**Materials and methods**

Eighty-eight faecal samples were collected from healthy dogs from two shelters from the North-East of Romania using sterile rectal swabs. The primary processing consisted of performing the E. coli ESBL producing screening using the Brilliance ESBL medium (Oxoid, Basingstoke, UK), a medium that contains cefpodoxime. The isolates that generated colonies characteristic of E. coli on the medium used for screening were molecularly confirmed as being E. coli based on the uidA and uspA genes (McDaniels et al., 1996; Anastasi et al., 2010) and phenotypically confirmed as being ESBL using the combination disc test (CDT) (MAST Group, UK). Also, all E. coli isolated on the Brilliance ESBL medium were tested for susceptibility to β-lactam and non-β-lactam agents through the diffusimetric method using Mueller-Hinton Agar (all disks and media from Oxoid, UK). The ATCC 25922 E. coli strain was used as control for the disk diffusion susceptibility test. The antimicrobials tested were as the follows: amoxicillin/clavulanic acid (30μg), ampicillin (10μg), aztreonam (30μg), imipenem (10μg), trimethoprim/sulfamethoxazole (25μg), enrofloxacin (5μg), tetracycline (30μg), chloramphenicol (30μg) and gentamicin (10μg).

Cell lysates obtained from all investigated isolates were screened by PCR for the presence of genes encoding for β-lactamase genes (bla\textsubscript{CTX-M}, bla\textsubscript{TEM}, bla\textsubscript{SHV}, bla\textsubscript{OXA}) and PMQR (qnrA, qnrB and qnrS) as previously described (Wedley et al., 2011; Dallenne et al., 2010; Robicsek et al., 2006).

A PCR scheme described by Clermont et al was used to assign the ESBL E. coli isolates to one of the 4 main phylogenetic groups (A, B1, B2, D). (Clermont et al., 2000)

**Results and discussions**

Eighty-eight faecal samples collected from the two shelters were analysed and screened for ESBL production. Results showed that, 28/88 (31.81%) ESC-resistant E. coli isolates were obtained and, of these, 21 isolates (75%) were phenotypically confirmed as extended-spectrum β-lactamase enzyme-producing strains. The antimicrobial susceptibility test was carried out for the 21 phenotypically positive ESBL isolates which demonstrated high levels of resistance to ampicillin (100% of them), amoxicillin/clavulanic acid (90.48%; 19/21); aztreonam (85.71%; 18/21) and tetracycline (47.62%; 10/21). In addition, 38.09% (8/21) of isolates were resistant to both enrofloxacin and trimethoprim/sulfamethoxazol whilst 14.29% (3/21) were resistant to chloramphenicol. All strains analysed using the diffusimetric test were susceptible to imipenem and chloramphenicol.

Molecular testing showed that bla\textsubscript{CTX-M} genes were identified in 17/28 (60.71%) of ESC-resistant E. coli isolates, 15 of which (88.24%) belonged to the CTX-M-1 group and only two (11.76%) to the CTX-M-9 group. No genes belonging to groups bla\textsubscript{CTX-M-2}, bla\textsubscript{CTX-M-9} and bla\textsubscript{CTX-M-25} were identified. In addition, 5/28 (17.86%) E. coli isolates carried bla\textsubscript{TEM}, and 4/28 (14.29%) carrying bla\textsubscript{SHV} were identified, while bla\textsubscript{OXA} was not identified in any ESC-resistant E. coli strain (table 1).

The investigations for the PMQR genes showed that 8/28 (28.57%) of the analysed isolates were positive, qnrS being the most prevalent gene, which was carried by 5/8 (62.5%) isolates; this was followed by qnrB, which was carried by 3/8 (37.5%) ESC-resistant E. coli (table 1). The qnrA was not identified for the analysed isolates.
In regard to the identified gene combinations, \( bla_{\text{CTX-M-1}} \) has been identified both in combinations with PMQR genes (\( qnrS, qnrB, \text{TEM} \)) and alone for 7 of the 15 carrying \( bla_{\text{CTX-M-1}} \) isolates (Table 1). Also, 4 of the 28 of E. coli analysed only harboured \( bla_{\text{TEM}} \) and \( bla_{\text{SHV}} \) gene combination.

Phylogenetic analysis showed that most ESC-resistant E. coli isolates belonged to the phylogenetic group A (12/28; 42.86%), followed by group B1 (10/28; 35.71%), B2 (4/28; 14.29%) and the phylogenetic group D (2/28; 7.14%).

All CTX-M enzymes are part of the extended-spectrum β-lactamase (ESBL) enzyme group, while the TEM and SHV enzymes, could be either β-lactamase or ESBLs (Ewers et al., 2011). Although we did not perform PCR product sequencing on the 4 of the 28 ESC-resistant E. coli isolates which carries \( bla_{\text{TEM}} \) and \( bla_{\text{SHV}} \) genes, phenotypic combination disc test (CDT) testing showed a typical ESBL phenotype in these isolates. Consequently, this study has identified a high prevalence (21/28; 75%) of extended-spectrum β-lactamase (ESBL) enzyme-producing E. coli isolates associated with the production of genes encoding the plasmid mediated resistance to quinolones, in the normal microbiota of healthy dogs.

Also, in our study, the most prevalent enzyme group was CTX-M-1, which was the most common enzyme identified in chicken, pets and also urban healthy dogs (Dahmen et al., 2012; Grami et al., 2013; Zurfluh et al., 2014; Haenni et al., 2014).

Although the number of analysed samples is small, the high prevalence of ESBL positive E. coli isolates identified in this study highlights the occurrence and likely circulation of these genes among healthy animals from shelters. This may also suggest that the unrestricted circulation of these dogs could facilitate the dissemination of these resistance isolates/genes in animal populations and between species.

Finally, the identification and characterisation of the main genes encoding ESBL enzymes in dog populations, stray or pets in Romania has epidemiological importance as this is the first study to evaluate their prevalence in this population. Knowledge of the sources and reservoirs for these genes is essential in order to reduce the risks of transmission to humans. In addition, better policies and guidelines for appropriate antimicrobial use are critical and need to be implemented in Romania to reduce the impact of selective pressure associated with antimicrobial use in veterinary medicine.

**Conclusions**

These findings indicate a high prevalence of ESBLs and PMQR associated resistance E. coli

<table>
<thead>
<tr>
<th>Source</th>
<th>bla gene combinations</th>
<th>No. of isolates/group</th>
</tr>
</thead>
<tbody>
<tr>
<td>dog</td>
<td>Total CTX (%)*</td>
<td>17/28 (60.71%)</td>
</tr>
<tr>
<td></td>
<td>CTX-M-1 group (%)</td>
<td>15/17 (88.24%)</td>
</tr>
<tr>
<td></td>
<td>CTX-M-1</td>
<td>7/15 (46.66%)</td>
</tr>
<tr>
<td></td>
<td>CTX-M-1, qnrS</td>
<td>4/15 (26.67%)</td>
</tr>
<tr>
<td></td>
<td>CTX-M-1, qnrS, TEM</td>
<td>1/15 (6.67%)</td>
</tr>
<tr>
<td></td>
<td>CTX-M-1, qnrB</td>
<td>3/15 (20%)</td>
</tr>
<tr>
<td>dog</td>
<td>Total CTX-M-9 group (%)*</td>
<td>2/17 (11.76%)</td>
</tr>
<tr>
<td></td>
<td>Total TEM (%)*</td>
<td>5/28 (17.86%)</td>
</tr>
<tr>
<td></td>
<td>TEM, SHV</td>
<td>4/28 (14.29%)</td>
</tr>
<tr>
<td>dog</td>
<td>Total SHV (%) *</td>
<td>4/28 (14.29%)</td>
</tr>
<tr>
<td></td>
<td>Total PMQR genes</td>
<td>8/28 (28.57%)</td>
</tr>
<tr>
<td></td>
<td>qnrS</td>
<td>5/8 (62.5%)</td>
</tr>
<tr>
<td></td>
<td>qnrB</td>
<td>3/8 (37.5%)</td>
</tr>
</tbody>
</table>

Note: * is the percentage of the screened gene present in any combination in the analysed groups (dogs)
in the normal microbiota of dogs from shelters, which carries the risk for dissemination of resistance genes to other animals, humans or the environment.

References


