Characterisation of Extended β -Lactamases and Plasmid Mediated Quinolones Resistance in *Escherichia Coli* from Shelter Dogs

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Bulletin UASVM Veterinary Medicine 76(1)/2019

Print ISSN 1843-5270; Electronic ISSN 1843-5378

doi:10.15835/buasvmcn-vm: 2019.0003

Abstract

The aim of this study 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 extended spectrum cephalosporin (ESC)-resistant *Escherichia coli* isolated from dog faeces from two shelters in the North-East of Romania. Eighty-eight faecal samples from healthy dogs were analysed by cultivation on Brilliance ESBL medium (Oxoid, UK), followed by phenotypic ESBL screening using combination disc test (CDT). Identification of the *E. coli* strains was performed by *uidA/uspA* gene PCR. Susceptibility testing was performed on Mueller-Hinton Agar, with β -lactam and non- β -lactam agents. Identification of β -lactamase genes (*bla*_{CTX-M}, *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA}) and PMQR genes (*qnrA*, *qnrB* and *qnrS*) was performed by PCR as previously described. Twenty eight ESC-resistant *E. coli* (31.81%) were obtained and (n=21/28, 75%) of these were confirmed as ESBLs and showed resistance to cefpodoxime (n=21/28, 75%), amoxicillin/clavulanic acid (n=19/21; 90.48%), and enrofloxacin (n=8/21; 38.09%). Predominant ESBL types were CTX-M-1 (n=15/17, 88.24%) and CTX-M-9 (n=2/17, 11.76%) enzymes. TEM and SHV enzymes were identified in 17.86% and 14.29% of the ESC-resistant isolates, whilst some isolates (n=4) carried only *bla*_{TEM} and *bla*_{SHV}. The prevalence of PMQR genes was 28.57% of the 28 ESC resistant isolates, consisting of *qnrS* (62.5%) and *qnrB* (37.5%). These findings indicate a high prevalence of ESBLs and PMQR associated resistance *E. coli* in the normal faecal microbiota of dogs from shelters, which carries the risk for dissemination of these resistance genes to other animals, human or the environment.

Keywords: CTX-M, PMQR, SHV, TEM, dogs

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 track 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 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 cefpodoximes. 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 follows: amoxicillin/clavulanic acid (30 μ g), ampicillin (10 μ g), aztreonam (30 μ g), imipenem (10 μ g), trimethoprim/sulfamethoxazole (25 μ g),

enrofloxacin (5 μ g), tetracycline (30 μ g), chloramphenicol (30 μ g) and gentamicin (10 μ g).

Cell lysates obtained from all investigated isolates were screened by PCR for the presence of genes encoding for β -lactamase genes (*bla*_{CTX-M}, *bla*_{TEM}, *bla*_{SHV}, *bla*_{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/sulfamethoxazole 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*_{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*_{CTX-M-2}, *bla*_{CTX-M-8}, and *bla*_{CTX-M-25} were identified. In addition, 5/28 (17.86%) *E. coli* isolates carried *bla*_{TEM}, and 4/28 (14.29%) carrying *bla*_{SHV} were identified, while *bla*_{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*_{CTX-M-1} has been identified both in combinations with PMQR genes (*qnrS*, *qnrB*, *TEM*) and alone for 7 of the 15 carrying *bla*_{CTX-M-1} isolates (table 1). Also, 4 of the 28 of *E. coli* analysed only harboured *bla*_{TEM} and *bla*_{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*_{TEM} and *bla*_{SHV} genes, phenotypic combination disc test (CDT) testing showed a typical ESBL phenotype in this 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 1. Prevalence and distribution of resistance gene combinations in *Enterobacteriaceae* strains isolated

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

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.

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