The Prevalence of ESBL-Producing Strains of *E. coli* and *K. pneumoniae*, Isolated from Pets Treated with Antibiotics – Preliminary Remarks

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Abstract

The prevalence of the strains of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae is continuously increasing at the global level. The appearance of ESBL enzymes represents a danger for the efficacy of treatments with beta-lactam antibiotics (Măciucă I., 2015). The aim of the study resided in assessing the prevalence of ESBL-positive strains of *E. coli* and *K. pneumoniae* in pets that were treated with antibiotics (Enrofloxacin, Ciprofloxacin, Cefadroxil) for various bacterial infectious diseases. In February 2015, 29 faeces samples were collected at the rectal level from dogs and cats. The samples were collected with the help of sterile buffers. For the screening of the strains of (ESBL)-producing Enterobacteriaceae, the Oxoid Brilliance chromogenic ESBL Agar medium was used, a specific medium for the isolation of (ESBL)-producing Enterobacteriaceae because it contains cefpodoxim, a second-generation cephalosporin to which all the extended-spectrum beta-lactamase (ESBL)-producing strains are resistant. The phenotypic confirmation of the isolated ESBL strains was achieved by using the combined disc method (Clinical and Laboratory Standards Institute, 2014). The taxonomic classification of the strains that were isolated was achieved by testing some minimal biochemical characteristics with the help of the MIU, TSI, EMBA, TBX tests. The *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 strains were used as a reference for quality control for the antibiotic sensitivity test. The results have been interpreted according to the CLSI 2014 standard. As a result of sample processing, we noticed a prevalence of 62.06% in the individuals who were carriers of *E. coli* and *K. Pneumoniae* ESBL-positive strains.

Keywords: *E. coli*, *K. pneumoniae*, ESBL

INTRODUCTION

The extended-spectrum beta-lactamase enzymes produced by Gram-negative bacteria (the *Enterobacteriaceae* family) represent a threat to public health in the human medicine and also in the veterinary medicine worldwide. Several studies have reported the transmission of zoonotic bacteria resistant to antibiotics between food-producing animals and humans, while the contribution of pets to this circuit is insufficiently known (Măciucă, 2015; Lucianne Leigue *et al.*, 2013). The social role of pets has changed throughout time so that a close relationship

was created between owners and themselves. Consequently, at the microbial level, between people and pets the horizontal transfer of bacteria carrying ESBL resistance genes may appear, genes that are located on mobile genetic elements (Carattoli *et al.*, 2008).

The administration of beta-lactam antibiotics (penicillins, cephalosporins) as a first choice in both the human and veterinary anti-infective therapy constitutes an important factor in the emergence of ESBL enzymes (EFSA Journal 2011; Cristina, 2012; www.who.com). Furthermore, the administration of fluoroquinolone antibiotics

(Ciprofloxacin) resulted in the emergence of in-cross resistance and implicitly to the emergence of extended-spectrum beta-lactamases (Axel, 2012). All these factors lead to the emergence of bacterial infections with limited therapeutic options and an increased risk of treatment failure.

The use of antibiotics in animals and humans, the direct contact and close cohabitation between pets and owners favors the spread of ESBL-producing bacteria (Ewers, 2011).

Most of the studies on ESBL-producing bacteria were performed on clinical isolates, thus ignoring the major reservoir of these bacteria, healthy animals (Paola Gandolfi-Decristophoris *et al.*, 2013). The aim of the study resided in assessing the prevalence of ESBL-positive strains of *E. coli* and *K. pneumoniae* in pets that were treated with antibiotics (Enrofloxacin, Ciprofloxacin, Cefadroxil) for various bacterial infectious diseases.

MATERIALS AND METHODS

The investigated material was represented by 29 faeces samples from clinically healthy dogs and cats presenting a medical history related to antibiotic treatments (Enrofloxacin, Ciprofloxacin, Cefadroxil).

After collection, samples were seeded on broth and incubated at 37°C for 24 h (Măciucă, 2014). A volume of 100 µl were taken from the liquid which was subsequently seeded on the Agar Oxoid ESBL Brilliance (Elise, 2013) solid chromogenic medium. This medium contains Cefpodoxime and is specific for isolating the strains of *Enterobacteriaceae* producing extended-spectrum beta-lactamases. According to the manufacturer's indications, the colonies of *Escherichia coli* are blue on this medium and those of *Klebsiella pneumoniae* are green (Livermoore, 2005; www.oxoid.com).

From the medium used for screening, the colonies obtained were transplanted on the TSA medium with a view to obtaining isolated colonies in order to subsequently achieve the taxonomic classification and phenotypic confirmation of presumptive ESBL strains (Măciucă, 2014).

The taxonomic classification of isolates was performed by transplanting the colonies obtained on polytrophe media. To this end, the colonies on the TSA medium were seeded on the MIU, TSI, EMBA and TBX medium, incubation occurring at 37° C for 24 hours, respectively at 44.5°C for 4

hours in order to identify the strains of *Escherichia coli*, biotype 1 (Măciucă, 2014).

The phenotypic confirmation of isolated strains was performed using the combined disc method. According to the CLSI standard 2014, the microtablets of cefotaxime (30 µg), cefotaxime/clavulanic acid (30/10 µg), ceftazidime (30 µg), ceftazidime/clavulanic acid (30/10 µg), cefpodoxime (10 µg) and cefpodoxime acid/clavulanic (10/10µg) were used (Clinical and Laboratory Standards Institute, 2014).

For each isolated strain, dilutions whose turbidity corresponded to the 0,5 McFarland turbidity were made. From the dilutions that were made in this way, 500 µl were inoculated on the Müller Hinton medium and microtablets were placed at a distance of 30 mm between them. Subsequently, incubating the plates with microtablets took place at 37 ° C for 24 hours. The interpretation of the results was performed using the CLSI standard 2014 so that a difference in diameter greater than 5 mm between cephalosporin and cephalosporin intensified by clavulanic acid confirms the phenotypic presence of extended-spectrum beta-lactamases for the strain tested (CLSI 2014; M'Zali *et al.*, 2000; Valentin *et al.*, 2014) (Fig. 1).

The strains of *E. coli* ATCC 25922 and *Klebsiella pneumoniae*, the ATCC 700603 (blaSHV-18) strain were used as reference within quality control for the test of antibiotic sensitivity of ESBL enzyme-producing strains (Rajkumar Manojkumar Singh *et al.*, 2014)

RESULTS AND DISCUSSION

After processing the data and obtaining the results, the prevalence was determined, prevalence which is itself a statistical indicator.

Out of the 29 samples of faeces, 24 (82.75%) generated cultures on the Brilliance ESBL Agar selective chromogenic medium, obtaining 26 strains of enterobacteria.

Following the phenotypic confirmatory test, out of the 26 strains, 20 (68.96%) were confirmed as being ESBL-producing strains. Moreover, we mention that, following the primary processing of samples, heteroresistant samples were also obtained.

Consequently, the prevalence of ESBL *Enterobacteriaceae* porting in the dogs and cats investigated was 62.06%.

Based on the minimal biochemical tests conducted, out of the 20 strains of *Enterobacteriaceae*, 17 (85%) were identified as *Escherichia coli* and 3 (15%) were identified as *Klebsiella pneumoniae* (Tab. 2, Fig. 3).

In a release in January 2015, the European Medicines Agency draws the attention on the impact of pets in the context of the interspecies transmission of multidrug-resistant bacteria. Furthermore, the importance of using microbial

Fig.1 The phenotypic confirmation of ESBL strains by using the combined disc method

Tab. 1. Results obtained after processing isolates

Collecting unit	Collected samples	Brilliance Esbl Agar samples	Negative samples	No. of strains obtained	No. of ESBL confirmed strains	Total no. of bearing individuals
Consultation rooms	29	24	5	26	20	18

Fig. 2. Synthesis of the results after processing samples

substances that are extremely important for human medicine in the anti-infective veterinary therapy is stressed, considering that this is an additional risk factor for the emergence and transmission of antimicrobial resistance (European Medicines Agency, 2015). In order to limit the emergence of ESBL enzymes, a strategic plan has to be developed so that the use of some important antibiotics for humans should be drastically limited in veterinary medicine.

The results are consistent with the literature concerning the occurrence of antimicrobial resistance after the administration of broad-spectrum cephalosporins and fluoroquinolones to pets (Damborg *et al.*, 2015).

In a study published in 2015, Cozma *et al.* obtained a prevalence equal to 25.86% of the *E. coli* and *K. pneumoniae* strains isolated from clinically healthy dogs that have not been treated with antibiotics.

Taking into consideration the fact that in Romania epidemiological data on antibiotic resistance through ESBL has not been reported and that the prevalence obtained by us is high, even in the conditions of a preliminary study, we

consider that extending the studies on a wider area and to a higher number of animals is necessary in order to determine the impact and role of healthy pets in transmitting bacteria carrying antibiotic resistance genes. We believe that such a study is necessary in the context in which One health one medicine is taken into consideration at a global level.

CONCLUSION

The prevalence of ESBL Enterobacteriaceae porting in clinically healthy dogs and cats that had a medical history related to antibiotic treatments, was 62.06%. The prevalence of *Escherichia coli* was 85% and that of *Klebsiella pneumoniae* was 15%.

Although the number of samples examined was small, the proportion of bacterial strains resistant to third generation cephalosporins registered in clinically healthy dogs and cats supports the development of a more comprehensive and detailed study in order to investigate the role of healthy pets in the circuit of the horizontal transmission of extended-spectrum beta-lactamase-producing enterobacteria.

Tab. 2. Taxonomic classification of isolated strains

Collecting unit	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	Total
Consultation rooms	17	3	20

Fig. 3. Results of the taxonomic classification of the strains obtained

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