

*Veterinarska i
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DOGS AS A SOURCE OF ANTIBIOTIC
RESISTANT *ESCHERICHIA COLI*

PSI KAO REZERVOAR SOJEVA
ESCHERICHIA COLI REZISTENTNIH NA
ANTIBIOTIKE

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Sažetak

Antibiotska rezistencija je pojava koja prati upotrebu antibiotika te dovodi do nastanka sojeva bakterija rezistentnih na antibiotike. Ovaj proces dešava se pod prirodnim uslovima, ali je u današnje vreme ubrzan neracionalnom upotrebom antibiotika. Kako se *Escherichia coli* zbog svoje specifične ekologije smatra markerom antibiotske rezistencije, a imajući u vidu i oskudne podatke o rezistenciji sojeva *E. coli* izolovanih od pasa s područja Republike Srbije, ali i sojeva *E. coli* uopšte, cilj ovoga istraživanja bio je da se utvrdi prisustvo antibiotske rezistencije kod sojeva izolovanih iz fecesa pasa. Ispitivanje antimikrobne rezistencije rađeno je Kirbi-Bauer metodom prema smernicama Instituta za kliničke i laboratorijske standarde i Evropskog komiteta za testiranje antimikrobne osjetljivosti. Većina izolata rezistentnih na antibiotike *E. coli* pokazalo je rezistenciju na ampicilin (22,5%), zatim na amoksicilin s dodatkom klavulanske kiseline i na cefuroksim (8,5%), na sulfametaksazol s dodatkom trimetoprima i na ciprofloksacin (7,5%), te na ceftriakson (2,5%). Svi ispitani izolati bili su osjetljivi na gentamicin. Nalaz *E. coli* izolata rezistentnih na većinu korišćenih antibiotika u ovom istraživanju ne samo da ima značaj za terapijski pristup u kliničkoj praksi malih životinja, već ima značaj i s aspekta javnog zdravlja u Srbiji.

INTRODUCTION

Antibiotics are the backbone of public health and play a major role in improving the health and well-being of humans and animals, whereby are used in the treatment of infections as well as in the prophylaxis and growth promotion of food-producing animals. Yet, there is the other side of the coin, while antibiotics have been successfully used in dealing with

infectious diseases, their use has grown exponentially leading to the emergence and spread of antibiotic resistance^(1, 2). Due to antibiotic selective pressure and selective pressure of the environment occurs the development of bacterial antibiotic resistance, with continued selective pressure with antibiotics used in routine therapy as a significant prerequisite for its emergence⁽³⁾.

The levels of antibiotic resistance which are found in commensal bacteria are considered as a good indicator for antibiotic selective pressure. As they represent a reservoir of resistance genes they point to resistance problems expected in pathogens (1). Due to its abundance and its biochemical and cultural characteristics *Escherichia coli* is often used in studies of bacterial resistance, and represents reliable indicator of antibiotic use in animals (4). Antimicrobial resistance among bacteria isolated from pets is a growing problem affecting public health and patient treatment, limiting therapeutic options and increasing the risk of therapy failure. Furthermore, the emergence of drug-resistant bacteria in dogs is a potential threat to human health. Results of many studies show that direct contact between pets and humans can lead to transmission of pathogenic bacteria from one to another, including strains that show antibiotic resistance (5).

As due to its specific ecology *E. coli* is considered as a marker of antibiotic resistance, and given the scarce data on the resistance of *E. coli* strains isolated from dogs from the territory of the Republic of Serbia, but also of *E. coli* isolates in general, the aim of this study was to determine the presence of antibiotic resistance in dog fecal isolates.

MATERIAL AND METHODS

This research included 80 strains of *E. coli*, isolated from dog faeces using standard bacteriological methods, and whose identity was determined by standard biochemical methods and confirmed matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) on Microflex LT automated system (Bruker Daltonik, Bremen, Germany). Antimicrobial susceptibility testing for *E. coli* isolates was performed by using the Kirby-Bauer method, the disk diffusion method respectively, according to the guidelines of the Institute for Clinical and Laboratory Standards and the European Committee on Antimicrobial Susceptibility Testing (6, 7). The antimicrobial susceptibility of all *E. coli* isolates was tested for the following antibiotics: ampicillin (10 µg), amoxicillin + clavulanic acid (20 + 10 µg), cefuroxime (30 µg), ceftriaxone (30 µg), gentamicin (10 µg), trimethoprim-sulfamethaxazole (1.25 + 23.75 µg), ciprofloxacin (5 µg). Isolates resistant to 3 or more antibiotic classes were considered multiresistant.

RESULTS AND DISCUSSION

Using disc diffusion method 24 of 80 (30%) *E. coli* isolates tested showed resistance to 1 or more antibiotics administered (Table 1). The majority of *E. coli* isolates showed resistance to ampicillin, 18 isolates respectively (22.5%), followed by amoxicillin with clavulanic acid and cefuroxime 7 (8.5%), trimethoprim-sulfamethaxazole and ciprofloxacin 6 (7, 5%) and ceftriaxone 2 isolates (2.5%). All the isolates tested were susceptible to gentamicin.

Table 1. Antibiotic resistance patterns of *E. coli* isolates

Isolate	Antibiotic						
	AMC	AMP	CXM	CRS	CN	SXT	CIP
13	R	R	S	S	S	S	S
14	S	R	S	S	S	S	S
23	R	R	S	S	S	R	S
28	R	R	S	S	S	S	S
33	S	R	S	S	S	S	R
34	S	R	S	S	S	R	S
39	S	S	S	S	S	S	R
41	S	R	S	S	S	S	R
42	S	R	S	S	S	R	S
44	S	R	R	S	S	S	S
46	S	R	S	S	S	R	S
48	S	R	S	S	S	S	S
56	S	S	R	S	S	S	S
74	S	R	S	S	S	S	S
78	R	R	S	S	S	S	I
79	S	S	R	S	S	S	S
81	S	S	R	S	S	S	R
86	R	R	R	I	S	R	I
87	S	R	S	S	S	S	S
88	S	S	R	S	S	S	S
91	S	S	R	I	S	S	S
93	S	R	S	S	S	S	S
97	R	R	S	S	S	R	S
98	R	R	S	S	S	S	S

Legend: AMC-amoxicillin + clavulanic acid, AMP-ampicillin, CXM-cefuroxime, CRO-ceftriaxone, CN-gentamicin, STX-trimethoprim-sulfamethaxazole, CIP-ciprofloxacin; R-resistant, I-intermediate, O- susceptible

One isolate (isolate 86) showed resistance to 4 classes of antibiotics, penicillins (amoxicillin + clavulanic acid and ampicillin), cephalosporins (II generation cefuroxime and III generation ceftriaxone), sulfonamides (trimethoprim-sulfamethaxazole) and fluoroquinolone (ciprofloxacin) respectively, and this isolate was declared as multidrug-resistant.

According to the available data, antimicrobial susceptibility of *E. coli* isolates originating from dogs was tested in only one study in Serbia. Only 5 isolates were included in the study, and one multidrug-resistant isolate was reported, with resistance to 7 of the 9 antibiotics tested. As in our study, resistance to antibiotics from the class of penicillins was reported, whereby the majority percentage of ampicillin over all antibiotics used, followed by sulfamethoxazole with trimethoprim and ciprofloxacin. The reporting of gentamicin susceptibility as well as the resistance of all ceftriaxone isolates in the above study is not in agreement with the results of our study (4). In regard to the class of antibiotics used in this study, the results of our research as well as of the other authors in the world indicate that *E. coli* isolated from dog

faeces developed the highest resistance levels to penicillins, whereby mostly to ampicillin. The percentage of isolates resistant to ampicillin and amoxicillin with clavulanic acid in this study is in agreement with the results reported by a number of authors, while these values are lower than those reported for *E. coli* isolated from faeces of dogs in Portugal and South Korea, but also significantly lower than those reported in Poland or in Brazil where 85.7% of ampicillin resistant isolates were reported (5, 8-14).

The finding of low resistance rates of *E. coli* isolates to the antibiotics from class of cephalosporins is in agreement with the results of studies reported in the United Kingdom, Portugal, Canada and Denmark (8, 9, 11, 14). Two groups of researchers, one from Brazil and the other in from the United States, reported a significantly higher percentage of isolates resistant to this class of antibiotics, with the highest resistance to cephalexin (33.3%) of strains isolated from dogs in Brazil (5, 12).

The low percentage of resistance to fluoroquinolones, i.e. to ciprofloxacin as representative of this class of antibiotics, obtained in our study is in agreement with the results of most other studies (9, 11-13). Contrary to these findings, Pedersen et al. in dogs in Denmark, as well as Murphy et al. in dogs in Canada, did not find isolates resistant to this antibiotic (8, 14).

Triptoprim-sulfometaxazole in this study was used as an indicator of *E. coli* resistance to sulfonamides, and the observed low percentage of resistant isolates is in agreement

with studies from Portugal and Canada, while other authors have reported a higher percentage of resistant isolates in dogs in the United States, South Korea, Brazil, Portugal, the United Kingdom, and Poland, in which this percentage reached 39.9% (5, 9-14).

In our study like in the study from Portugal, none of the *E. coli* isolates showed resistance to gentamicin, which was used as an indicator of resistance to aminoglycosides (9). Findings of one gentamicin resistant isolate, and thus very low resistance, have been reported in dogs in Denmark and Canada, while the authors of several studies on susceptibility of *E. coli* isolated from dogs in Brazil, the United States, Poland and South Korea report a significantly higher percent gentamicin-resistant isolates reaching up to 68.1% (5, 8, 10, 12-14).

CONCLUSION

Evidence of the sharing of *E. coli* clones with the same antibiotic resistance patterns between dogs and their owners indicates interspecies transmission, in other words pose the risk for humans of acquiring antibiotic-resistant isolates from dogs, and vice versa (Stenske et al., 2009). Therefore, the finding of *E. coli* isolates resistant to the majority of used antibiotics in this study not only has relevance to the therapeutic approach in the clinical practice of small animals, but also has an impact on public health in Serbia.

Abstract

Antibiotic resistance is a phenomenon that follows the use of antibiotics, resulting in the emergence of antibiotic-resistant strains of bacteria. This process occurs naturally but nowadays it is accelerated by irrational use of antibiotics. As due to its specific ecology *E. coli* is considered as a marker of antibiotic resistance, and given the scarce data on the resistance of *E. coli* strains isolated from dogs from the territory of the Republic of Serbia, but also of *E. coli* isolates in general, the aim of this study was to determine the presence of antibiotic resistance in dog fecal isolates. Antimicrobial susceptibility testing for *E. coli* isolates was performed by using the Kirby-Bauer method according to the guidelines of the Institute for Clinical and Laboratory Standards and the European Committee on Antimicrobial Susceptibility Testing. The majority of *E. coli* antibiotic-resistant isolates showed resistance to ampicillin (22.5%), followed by amoxicillin with clavulanic acid and cefuroxime (8.5%), trimethoprim-sulfamethaxazole and ciprofloxacin (7, 5%) and ceftriaxone (2.5%). All the isolates tested were susceptible to gentamicin. The finding of *E. coli* isolates resistant to the majority of used antibiotics in this study not only has relevance to the therapeutic approach in the clinical practice of small animals, but also has an impact on public health in Serbia.

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