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The Characterization of *Escherichia coli* Strains Isolated from Urinary Tract Infections in Cats and Dogs[*]

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Abstract: *The aims of the study were to perform phylogenetic grouping, to detect the virulence associated genes in Escherichia coli strains recovered from the urinary tract infections of dogs and cats, and to determine the antibiotic susceptibility profiles, as well. The urine samples from the animals showing clinical signs of urinary tract infection examined for the occurrence of E. coli. Phylogenetic classification of the isolates was performed according to the existence of chuA, yjaA and TSPE4.C2 genes. The isolates were also examined for the presence of uropathogenecity-associated genes, such as, fimH, sfa1, iut, fyu, hly, cnf-1, papG and for the diversity of the papG allelles. Finally, the antimicrobial susceptibility profiles were examined. E. coli was isolated from 11 (6.08%) of 181 samples. Two isolates belonged to group A-2, while the remaining isolates belonged to group B-2. hylA was detected in 2 (25%); sfa1, cnf1, papG and papGIII were detected in 3 (37.5%); iutA was detected in 4 (50%); fyuA was detected in 6 (75%); and fimH was detected in all of the isolates. Most of the isolates were resistant to ampicillin while one isolate also showed multidrug resistance. The isolation rate of E. coli was low in this study, however, we had a brief data about the phylo-groups and virulence factors of canine and feline uropathogenic strains.*

Keywords: Antibiotic resistance, Cats, Dogs, Phylo-typing, Uropathogenic *Escherichia coli*, Virulence factors.
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Üriner Sistem İnfeksiyonlu Kedi ve Köpeklerden İzole Edilen *Escherichia coli* Suşlarının Karakterizasyonu

Öz: *Bu çalışmada kedi ve köpeklerde üriner sistem infeksiyonlarından izole edilen E. coli izolatlarının filogenetik tiplendirmesinin yapılması, virulansla ilişkili genlerinin saptanması ve antibiyotiklere duyarlılıklarının araştırılması amaçlanmıştır. Bu amaçla üriner sistem şikayeti olan kedi ve köpeklerden idrar örnekleri toplandı ve E. coli yönünden incelendi. İzolatların filogenetik sınıflandırması chuA, yjaA ve TSPE4.C2. gen bölgelerinin varlığına göre yapıldı. Bunu takiben tüm izolatlar üropatojenite ile ilişkili faktörlerden fimH, sfa1, iut, fyu, hly, cnf-1, papG varlığı ve papG allellerinin dağılımı yönünden incelendi. Son olarak izolatların antibiyotiklere duyarlılıkları belirlendi. İncelenen 181 idrar örneğinin 11 (% 6,08)'inden E. coli izole edildi. Filogenetik tiplendirme sonucunda izolatların ikisinin A-2 geri kalan altısının B2-2 filogenetik sınıfa ait oldukları saptandı. İzolatların 2 (%25)'sinde hlyA; 3 (%37.5)' ünde sfa1, cnf1, papG ve papGIII; 4 (%50)'ünde iutA; 6 (%75)'sında fyuA ve tümünde fimH saptandı. İzolatların çoğunluğunu ampisiline dirençli olduğu, bir izolatın çoklu direnç gösterdiği saptandı. E. coli izolasyon oranı az olmakla birlikte, bu çalışma sonucunda kedi ve köpeklerdeki üropatojen suşların filogenetik grupları ve virulans faktörleri hakkında ön bilgi elde edildi.*

Anahtar sözcükler: Antibiyotik direnç, Filogenetik tiplendirme, Kedi, Köpek, Üropatojen *Escherichia coli*, Virulans faktör.
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GİRİŞ

Escherichia coli is a normal commensal inhabitant of the intestines of humans and warm-blooded animals. This colonization of the bacteria occurs during the first days of life. Enterotoxigenic and enteropathogenic strains of *E. coli* are the most famous groups causing gastrointestinal diseases. However, some strains cause bacteraemia, pyometra, vaginitis and urinary tract infections and those strains are called extra-intestinal pathogenic *E. coli*. Among them, the most common group is uropathogenic *E. coli*

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(UPEC) (Yuri et al., 1998; Oluoch et al., 2001; Chen et al., 2003; Tramuta et al., 2011). *E. coli* originated urinary tract infections (UTI) are one of the commonly seen health problems of companion animals. Furthermore, by the increase in the prevalence of antibiotic resistant *E. coli* strains, treatment procedures of UTI became more complicated (Oluoch et al., 2001; Çetin et al., 2003; Drazenovich et al., 2004; Thompson et al., 2011).

E. coli strains can be divided into four main phylogenetic groups; A, B1, B2, D. Extra-intestinal *E. coli* strains are commonly derived from group B2 and D. The strains in group B2 contain a high number of virulence factors, while the ones in group D have fewer factors. However, strains with a low number of virulence factors belonging to group A and B1 can cause disease particularly in immunocompromised hosts and they can be pathogenic in healthy ones if they acquire sufficient virulence factors (Clermont et al., 2000; Tramuta et al., 2011). Recently, with multi-locus sequence data and genome data, eight phylo-groups (A, B1, B2, C, D, E, F and clade 1) have been recognised (Clermont et al., 2013). Type 1 fimbriae (*fim*), pilus associated with pyelonephritis (*pap*), S fimbria (*sfa*), afimbrial adhesion (*afa*), α- haemolysin (*hly*), aerobactin, cytotoxic necrotizing factor 1 (*cnf1*), yersiniabactin (*fyuA),* outer membrane protease *(ompT*) are the common virulence factors of UPECs recovered from cats and dogs (Yuri et al., 1998; Johnson et al., 2003; Drazenovich et al., 2004; Tramuta et al., 2011).

The aims of this study were to characterize the *E. coli* strains recovered from urinary tract infections from cats and dogs; to perform phylogenetic grouping and to detect the virulence associated genes and antibiotic susceptibility profiles of isolates.

MATERIAL and METHODS

108 cats and 73 dogs were referred to Department of Internal Medicine, Faculty of Veterinary Medicine with one or more urinary clinical signs (stranguria, haematuria, pollakiuria, inappropriate urination, excessive leaking of the genital area). All the animals were physically examined and their anamnesis were recorded. The urine samples were collected by ultrasound-guided cystocentesis. The animals were added to the study, which had no antimicrobial or anti-inflammatory treatment within last 10 days. All urine samples were sent to the Department of Microbiology for bacteriological examination. All studies in the study were carried out in line with Guide for the Care and Use of the Laboratory Animals principles and animal rights were protected (ethics committee approval number: 2013-119).

Urine samples were diluted to 1:10 with Nutrient broth and inoculated onto Nutrient Agar (HiMedia M001, HiMedia Laboratories Pvt. Ltd, Mumbai, India) supplemented with 7% sheep blood and MacConkey Agar (CM115, Oxoid, Cambridge, UK) plates, incubated at 37°C for 24 hours under aerobic condition. Colonies on plates were counted and cultures were evaluated as positive if they yielded $\geq 2x10^2$ CFU/ml. Gram negative bacilli were examined by conventional biochemical tests for identification of *E. coli* (Quinn et al., 1999; Oluoch et al., 2001; Johnson et al., 2003). Following the initial identification, all the isolates were further analysed by PCR for the presence of *uidA* housekeeping gene region (Tsai et al., 1993). For this purpose, the bacterial DNA was prepared as described previously by Kariyama et al. (2000) and PCR were performed as described by Tsai et al. (1993). PCR products were analysed on 2% agarose gel stained with ethidium bromide (0.5 µg/ml, Bio Basic Inc.) for the presence of 147 bp bands. All the samples were examined for other aerobic bacteria species by conventional methods (Quinn et al., 1999).

Antimicrobial susceptibilities of the isolates were detected by disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) standards with the antibiotic discs (Oxoid); amikacin (30 µg), ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), cefixime (5 µg), cephalexin (30 µg), enrofloxacin (5 µg), gentamicin (10 µg), nitrofurantoin (300 µg), difloxacin (30 µg), sulfamethoxazole-trimethoprim (23.75/1.25 µg), tetracycline (30 µg), ceftiofur (30 µg), marbofloxacin (5 µg), meropenem (10 µg) (NCCLS, 2004; CLSI, 2013).

Extended spectrum beta lactamase (ESBL) producing E. coli screening test performed with cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefpodoxime (10 µg), aztreonam (30 µg) discs according to CLSI standards (CLSI, 2013). The isolates were evaluated as multidrug resistant if they are intermediately susceptible or resistant to three or more antimicrobial classes. E. coli ATCC 25922 was used as a quality control.

Phylogenetic grouping were performed by multiplex PCR for *chuA, hjaA* genes and DNA fragment of TSPE4.C2 and the results were evaluated according to diagram described previously by Clermont et al. (2000). Each of the isolates was examined for the presence of the virulence-associated genes such as adhesins, siderophores and toxins. Briefly, Type-1 fimbria, (*fimH),* pyelonephritis associated P fimbria (*pap*) and pap alleles, S/F1C fimbria (*sfa/foc*), aerobactin (*iutA*), Yersinia siderophore receptor (fyu*A*), haemolysin (*hlyA*), cytotoxic necrotizing factor 1 (*cnf-1*) were amplified by PCR with the primers described previously (le Bouguenec et al., 1992; Yamamoto et al.,

1995; Johnson et al., 1996; Johnson et al., 2000a). E. coli strains (EcoR20, EcoR58, EcoR48 and EcoR62, B-1-5-1, JJ079, JJ338, JJ055) kindly provided by Prof. Dr. James R Johnson from University of Minnesota were used as positive control strains in PCR.

RESULTS

E. coli was isolated from 11 (5 cats, 6 dogs) of 181 animals and *uidA* genes were detected in all of the isolates. *E. coli* isolation rate was 6.08%. In the first year of the study, the freezer has been broken down due to electricity problem and all of the strains in the freezer were melted and it was impossible to use of any strains. Therefore, three isolates were excluded from the study, all the following results have been given for 8 *E. coli* isolates except total isolation rate.

Most of the isolates were resistant to ampicillin, difloxacin, enrofloxacin and tetracycline resistance followed this by respectively. ESBL was not detected in any *E. coli* isolates. Antimicrobial susceptibilities of the isolates were shown in Table 1.

According to phylogenetic analysis, 2 isolates belonged to group A, while the remaining 6 belonged to group B-2. The *fimH* gene was detected in all isolates and the *fyuA* gene was detected in 6 isolates, all belonging to group B-2. The *iutA* gene was detected in 50% of the isolates. The isolates belonging to B2 group carried a higher number of virulence factors (Table 2).

Table 1. Antimicrobial susceptibility results of the isolates

Isolate code	Antimicrobial agent													
	DIF	AM	SXT	AK	FUR	GM	AMC	ENO	MAR	TE	MEM	CFM	CL	F
U-1	S	**I**	S	S	**I**	S	S	S	S	S	S	S	S	S
U-2	**R**	**I**	S	S	S	S	S	**R**	**R**	**R**	S	S	S	S
U-3	**I**	S	S	S	S	S	S	**I**	S	S	S	S	S	S
U-4	S	**I**	S	**I**	S	**I**	S	S	S	S	S	S	S	**I**
U-5	S	**R**	S	S	S	S	S	S	S	**I**	S	S	S	S
U-6	S	**R**	S	S	S	S	S	S	S	S	S	S	S	S
U-7	S	**R**	S	S	S	S	S	S	S	**I**	S	S	S	S
U-8	**R**	**R**	S	S	S	S	S	**R**	**R**	S	S	S	S	S

S= Susceptible, I= Intermediate susceptible, R= Resistant, AK= amikacin (30 µg), AM=ampicillin (10 µg); AMC= amoxicillin/clavulanic acid (20/10 µg), CFM= cefixime (5 µg), CL= cephalexin (30 µg), ENO= enrofloxacin (5 µg), GM= gentamicin (10 µg), F= nitrofurantoin (300 µg), DIF= difloxacin (30 µg), SxT= sulfamethoxazole-trimethoprim (23.75/1.25 µg), TE= tetracycline (30 µg), FUR= ceftiofur (30 µg), MAR= marbofloxacin (5 µg), MEM= meropenem (10 µg)

Table 2. Phylogenetic analysis and distribution of the virulence factors of the isolates

Isolate Code		U-1	U-2	U-3	U-4	U-5	U-6	U-7	U-8
Phylogenetic group		A	A	B2	B2	B2	B2	B2	B2
Virulence Factors	*sfa-1*	-	-	+	+	-	+	-	-
	fimH	+	+	+	+	+	+	+	+
	hlyA	-	-	-	+	-	+	-	-
	cnfl	-	-	+	-	-	+	+	-
	fyuA	-	-	+	+	+	+	+	+
	iutA	+	+	-	+	-	-	-	+
	pap3	-	-	+	-	-	+	+	-
	papGI	-	-	-	-	-	-	-	-
	papGII	-	-	-	-	-	-	-	-
	papGIII	-	-	+	-	-	+	+	-

DISCUSSION and CONCLUSION

Urinary tract infection (UTI) is one of the common health problems in companion animals. Pathological disorders comprised after the infection can cause prostatitis, pyelonephritis, septicaemia, etc. Various microorganisms, of which, the most common species are *E. coli, Proteus* spp., *Pseudomonas* spp., *Staphylococcus* spp., and *Streptococcus* spp. can cause UTI (Yuri et al., 1998; Johnson et al., 2000a; Oluoch et al., 2001; Çetin et al., 2003; Drazenovich et al., 2004). Oluoch et al. (2001) isolated *E. coli* from 89% of the urine specimens. Çetin et al. (2003) examined 100 urine samples from dogs and observed bacterial growth in 38 of them, and *E. coli* were isolated from 12 animals. Eggertsdóttir et al. (2007) reported bacterial growth in 44 (33%) of the 134 cats examined with signs of lower urinary tract disorders. Lund et al. (2015) obtained a total of 82 bacterial isolates from 71 cats with lower urinary tract disease. They reported that *E. coli* was the most common bacterium with 38.8% isolation rate followed by *Staphylococcus* spp. (11%), *Enterococcus* spp. (7%). Hariharan et al. (2016) bacteriologically investigated 151 canine urine samples and reported that no bacterial growth was observed in 83 of them. Out of 52 culture positive samples, the most frequent species were *E. coli* (26.9%), *Proteus mirabilis* 18.2%, and *Staphylococcus intermedius* (14.6%). In the current study, *E. coli* was isolated from 11 (6.08%) samples out of 181 samples. Bacterial growth was observed in 38 (20.99%) animals. The low isolation rate of *E. coli* might be due to the theraphy protocols which had been applied to the animals before coming to the veterinary faculty's clinics. Other bacterial species were isolated as *Staphylococcus* spp., *Enterobacter cloacae*, *Enterococcus* spp., *Proteus* spp., *Streptococcus* spp., *Citrobacter diversus* and *Aerococcus viridans* from urine samples (data was not shown). When we compared the rate of *E. coli* isolation according to the samples which had bacterial growth, the rate was 28.95% (11 isolates out of 38).

Oluoch et al. (2001) detected gentamicin, amikacin, norfloxacin, enrofloxacin as the most effective antibiotics, and amoxicillin, carbenicillin, ampicillin, tetracycline and cephalothin as the less effective antibiotics against *E. coli* which caused UTIs. Similarly, Çetin et al. (2003) reported amoxicillin/clavulanic acid, gentamicin, enrofloxacin and danofloxacin as the most effective antibiotics. They mostly detected cephalothin resistance in *E. coli* strains. Babacan et al. (2011) reported the resistance for cephalothin and sulfamethoxazole/ trimethoprim were common in *E. coli* isolates from UTIs. The authors couldn't detect any resistance for gentamicin and cephalexin. Chang et al. (2015) detected resistance particularly for ampicillin and oxytetracycline. Oluoch et al. (2001) reported as gentamicin, norfloxacin, amikacin, enrofloxacin had the highest efficacy

against *E. coli* isolates from different samples but mostly urine samples. Çetin et al. (2003) found amoxicillin/clavulanic acid, gentamicin and ampicillin/sulbactam as the most effective antibiotics. In the current study, a less effective antibiotic was ampicillin, only one isolate was susceptible to this antibiotic. The resistances were followed by difloxacin, enrofloxacin and tetracycline, respectively. The resistances of gentamicin, amikacin, and cephalexin were low, similar to previous studies. ESBL, which is common and important for the public health, was absent. Fluoroquinolones are the common antibiotics used in companion animals and in this study, two isolates were resistant to all antibiotics in this group, furthermore one of those isolates was multidrug resistant.

Uropathogenic *E. coli* strains can be different from commensal intestinal strains by their phylogenetic groups and virulence factors (Emödy et al., 2003). *E. coli* strains fall into four main phylogenetic groups (A, B1, B2, and D) and virulent extra-intestinal strains mostly take place in group B2 and D (Clermont et al., 2000; Günaydın et al., 2017). Tramuta et al. (2011) examined *E. coli* strains obtained from dogs and cats with cystitis, and reported that 65% of the isolates belonged to group B2, 10% to group D, 15% to group B1, and 10% to group A. The authors indicated that strains belonging to group B harbored more virulence factors compared to the isolates from other groups. Johnson et al. (2001; 2003) found that most of the canine *E. coli* isolates were from group B2 and D. Wells et al. (2013) reported that 42% of the 159 canine isolates were from group B2, carried highest the number of virulence factors. In the present study, two isolates belonged to group A, while the remaining 6 to group B2. And it was particularly clear that the isolates belonging to B2 group carried more number of virulence factors. Clermont et al. (2013) improved the phylo-typing method of *E. coli* in 2013 and determined eight phylo-groups. The authors suggested that there is a little need to retest the isolates determined as group B1 or B2, however strains assigned to group A and D should be retested. In our study, only two strains were assigned to group D. Due to the low number of isolates and limitation of the project, those strains could not have re-tested by the new method.

Urovirulent *E. coli* strains harbour one or more number of virulence factors: afimbrial adhesin I (*afa*1), aerobactin system, cytotoxic necrotizing factor 1(*cnf*-1) or factor 2, (*cnf1 or cnf 2),*α- haemolysin (*hly*), pyelonephritis associated fimbria (P fimbria), S fimbria (*sfa*), *Yersinia* associated siderophore *Yersinia* bactin (*fyu*A) (Yuri et al., 1998; Johnson et al., 2000a; Chen et al., 2003; Emödy et al., 2003; Drazenovich et al., 2004; Wells et al., 2013). Yuri et al. (1998) reported virulence factors such as *pap, fim, sfa, hlyA, cnf1* were significantly higher in *E. coli* strains from dogs with cystitis, while the positive rates of those virulence factors were similar in uropathogenic and faecal isolates from cats. Tramuta et al. (2011) detected *fim*A gene the most commonly, *sfa* and *cnf*1 were following this. Drazenovich et al. (2004) detected *cnf, hly* and *sfa* from dogs with signs of chronic UTI. Johnson et al. (2003) found 30 different virulence factors in canine *E. coli* isolates from UTI and reported that *papG, pap allel III, sfa/foc, hlyA, fyuA, ompT* were the most common factors. The authors suggested that those factors could have been associated with uro-virulence. Siqueira et al. (2009) detected *cnf-1, iucD, hlyA, sfaS, papC, papGIII, usp* and *afa* in *E. coli* isolates from dogs. Wells et al. (2013) isolated 159 *E. coli* strains from canine urinary tract diseases and reported the most commonly virulence factors were *sfa* (33%), *cnf* (25%), *hly* (24%) and *pap* (18%). In this study, *fimH* gene encoding the subunit of mannose specific adhesin was detected in all of the isolates similar to reports of many researchers. This fimbria is important due to its ability to promote bacterial adhesion, invasion and biofilm production (Emödy et al., 2003). Some researchers indicate that there is not distinct correlation between uropathogenecity and this fimbria (Yuri et al., 1998; Chen et al., 2003), but since in this study we only examined low number of strains only from urinary tract infections, it is not possible to make any comments on this. Nevertheless, the *fimH*-encoded fimbria could have supported the bacteria in group A2 to produce urinary tract infections. The two following virulence factors detected were siderophores (*fyuA* and *iutA*) with prevalence of 75% or 50%, respectively. Virulence associated factors were detected mostly in 6 isolates belonging to phylogenetic group B2. Only *fimH* and *iutA* were detected in isolates belonging to group A2.

P-fimbria is another important virulence factor on bacterial adhesion and colonization. Some studies show that *E. coli* strains carrying *pap*-gene recovered from urinary tract infections or pyometra usually have *papGIII* allele. Some researchers indicated that *papG III*, is a predominant *papG* allele among *E. coli* isolates from humans with cystitis or pyelonephritis (Johnson et al., 2000b; Chen et al.,2003; Emödy et al., 2003; Wells et al., 2013; Günaydın et al., 2017). Johnson et al. (2001) reported 59% of canine *E. coli* isolates expressed *papGIII* allel. In this study, similarly, three isolates carrying *papG* genes had *papGIII* allel.

It is important to distinguish uropathogenic *E. coli* from other *E. coli* strains in terms of proper treatment. The identification of virulence factors carried by the uropathogenic *E. coli* species, as well as the ongoing collection of epidemiological data, especially in companion animals, is also important in terms of public health. Since treatment of UTI is getting more difficult and complicated

due to the increase of antibiotic resistance such studies should be continued and national data should be recorded.

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