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Detection of virulence factors of *Escherichia coli* strains isolated from urogenital system infections in dogs and cats**

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ABSTRACT:

In this study, virulence factors such as serum resistance, aerobactin iron uptake systems, adhesins (type 1 fimbria, P fimbria, S fimbria, afimbrial adhesins), haemolysine, uropathogenic spesific protein and cytotoxic necrotizing factor in 45 Escherichia coli isolates from dogs and cats with urinary tract and genital system infections, were investigated by both phenotypic methods and molecular methods. In PCR examinations of 45 E. coli, hlyA gene was detected in 73.3% of all isolates. fimH was found in 100%, papC in 71.1%, sfaDE in 82.2%, and afaBC in 0% of the isolates. iucD, traT, usp, cnf1 and cvaC genes were found in 20%, 71.1%, 84.4%, 75.5%, and 11.1% of the isolates, respectively. According to the isolation rate of hlyA and haemolytic activity on blood agar, this virulence factor is commonly seen in these strains and important in these infections especially pathogenesis. İsolates was found to be a high rate positive for usp gene. This result suggested that this strain may have higher infectivity. Cytotoxic necrotizing factor was common and important virulence factor in uropathogenic E. coli and prognosis of infection. However, colisin encoding gene (cvaC) only were detected from 5 of urine samples from dogs. This virulence factor could be important for the urinary tract infections in dogs rather than cats. Haemolysis, type 1 fimbriae, P fimbriae, S fimbriae, cnf, serum resistance, uropathogenic specific protein were detected at high rates by molecular method. In *E. coli* strains isolated from dogs, virulence factors were found to be higher than cat isolates.

*Kedi ve köpeklerin ürogenital sistem infeksiyonlarından izole edilen Escherichia coli'lerin virulens faktörlerinin belirlenmesi***

ÖZET:

Bu çalışmada, köpek ve kedilerin idrar yolu ve genital sistem enfeksiyonlarından izole edilen 45 Escherichia coli izolatlarında serum direnci, aerobaktin demir alım sistemleri, adezinler (tip 1 fimbria, P fimbria, S fimbria, afimbrial adezinler), hemolizin, üropatojenik spesifik protein ve sitotoksik nekrotizan faktör gibi virülans faktörleri hem fenotipik yöntemler hem de moleküler yöntemler ile araştırıldı. 45 E. coli izolatının virulens genlerine ait PCR sonuçlarında, tüm izolatların % 73.3'ünde hlyA geni tespit edildi. İzolatların% 100'ünde fimH,% 71.1'inde papC, % 82.2'sinde sfaDE ve % 0'ında afaBC bulundu. iucD, traT, usp, cnf1 ve cvaC genleri, izolatların sırasıyla% 20,% 71.1,% 84.4,% 75.5 ve % 11.1'inde bulundu. HlyA'nın izolasyon oranı ve kanlı agarındaki hemolitik aktivitesine göre, bu virülans faktörü bu suşlarda yaygın olarak görüldüğü ve bu enfeksiyonlarda özellikle patogenezde önemli olduğu düşünüldü. İzolatlarda usp geni için yüksek oranda pozitif olduğu bulunmuştur. Bu sonuç, bu bakterilerin daha yüksek bir enfektiviteye sahip olabileceğini düşündürmektedir. Sitotoksik nekrotizan faktör üropatojenik E. coli ve enfeksiyon prognozu için yaygın ve önemli virülans faktörüdür. Bununla birlikte, köpeklerden idrar numunelerinin sadece 5'inde kolisin kodlayan gen (cvaC) tespit edildi. Bu virülans faktörü, kediler yerine köpeklerde idrar yolu enfeksiyonları için önemli olabilir. Hemoliz, tip 1 fimbria, P fimbria, S fimbria, cnf, serum direnci, üropatojenik spesifik protein moleküler yöntemle yüksek oranlarda saptandı. Köpeklerden izole edilen E. coli suşlarında, virülans faktörlerinin kedi izolatlarından daha yüksek olduğu bulundu.

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1. Introduction

Urinary tract infections are mostly caused by bacteria. *E. coli* is the most common pathogen isolated from urinary tract infections (20, 24, 25, 28, 32, 36). *E. coli*, which causes urinary tract infections, is called as uropathogenic *E. coli* (UPEC) (23) or extraintestinal pathogenic *E. coli* (ExPEC) and these strains can be found in intestinal flora (37).

UPEC causes various diseases as opportunistic pathogens in humans and animals, especially in cats and dogs. These bacteria can also cause vaginitis, pyometra especially in femeale dogs rather than female cats (2, 4, 6) cystitis and pyelonephritis (4, 39). *E. coli* is the most isolated (62-90%) bacteria in pyometra. This predominance may be caused by the presence of *E. coli* in the normal vaginal flora and entry into the uterus through proestrus and oestrus. *E. coli* attaches to specific receptors in the endometrium stimulated with progesterone and is important for this infection. *E. coli* can be a causative agent for pyometra infection in dogs with subclinical urinary tract infection simultaneously (16). Virulence factors such as lipopolysaccharide and fimbria of bacterium cause the activation of epithelial cell receptors (37). These UPEC strains has vaious virulens factors such as adherence factors, aerobactin, hemolysin, cytotoxic necrotizing factor (cnf), S fimbria (sfa), pilus associated with pyelonephritis gene (pap) and uropathogenic specific protein (usp), which are play a role occurrence of infection (12, 34). In addition, some researchers have investigated colicine and serum resistance properties (12, 17, 19). Uropathogenic spesific protein (usp) may contribute for *E. coli* infectivity, and was detected more often in UPEC strains than in fecal *E. coli* strains obtained from healthy human and animals (34). hlyA (α-hemolysin), destroy to erythrosites (7, 10), penetrates nucleated cells and invade mucosal barriers, causes damage to the immune system, uses the nutrient and iron of the host cell. At low doses, it causes damage to the bladder and kidneys. Uropathogenic *E. coli* strains with hemolytic properties usually synthesize in P fimbria (10).

The production of hemolysis in patients with pyelonephritis, cystitis and asymptomatic bacteriuria is associated with uropathogenic *E. coli*. cnf-1 provides the mechanism that allows E.coli to invade kidney cells. In addition, it increases virulence and inflammatory response of the host cell in the pathogenesis of urinary tract infections. In humans, usp is more common in UPEC isolates than in fecal E. coli isolates. Many studies have shown several roles for usp in the pathogenesis of urinary tract infections in different urinary tract infection syndromes and patient groups (10). Rijavec et al. (31) reported that there is a meaningful relationship between usp and urinary tract bacteremia suggesting that UPECs are important in the transition from the urogenital system to the bloodstream. Other studies have shown the comparable prevalence of usp in cystitis, pyelonephritis and prostatitis isolates. Also, there is often a relationship between usp and all common serotypes of UPEC (10).

In order for UPEC to survive in the urinary tract with limited iron, UPEC demonstrates the ability to increase the number of receptors on the cell surface (upregulation) of the host required for the intake of iron-producing small molecule iron chelators. Studies have shown a relationship between aerobactin and P fimbria in patients with urinary tract infection and urosepsis (10).

Fimbriae connect to host tissues and provide colonization of the agent. Fimbria can be determined by in-vitro tests by taking advantage of their binding properties to erythrocytes. According to whether the hemagglutination feature is lost in the presence of D-mannose in the environment, fimbriae can be classified as mannose sensitive (type 1 fimbria) and mannose resistance (P fimbria, S fimbria and afimbrial adhesins) adhesins (8). Type 1 fimbria facilitates the survival of bacteria, stimulation of mucous inflammation, bacterial invasion, bacterial growth and biofilm production. Many studies confirm that type 1 fimbria plays a vital role in bladder colonization (10).

P fimbriae allow early infections of epithelial cells of renal tubules by UPEC; type 1 fimbriae increase infection of the tubule center through bacterial attachment and biofilm formation. These cause complete blockage of the nephron and ineffective kidney filtration that causes the disease state called pyelonephritis. S fimbria is associated with the spread of sepsis, meningitis and increased urinary tract infections caused by *E. coli*. Afa adhesins are associated with urinary tract infections caused by E. coli, especially acute gestational pyelonephritis and recurrent cystitis. The findings of the studies suggest that there is a possible role for Dr / Afa adhesins in recurrent or chronic urinary tract infections. (10).

There is limited study in the world about prevalance of virulence factors in E.coli strains isolated from urinary tract and genital system infection in dogs and cats.

In this study, virulence factors such as serum resistance, aerobactin iron uptake systems, adhesins (type 1 fimbria, P fimbria, S fimbria, afimbrial adhesins), haemolysine, uropathogenic spesific protein and cytotoxic necrotizing factor in 45 *E. coli* isolates from dogs and cats with urinary tract and genital system infections, were investigated by both phenotypic methods and molecular methods.

2. Material and Methods

Sampling:

The samples were obtained from Ankara University Faculty of Veterinary Medicine Clinics and private pet hospitals between 2009 and 2010, which had been sent to Ankara University Faculty of Veterinary Medicine Department of Microbiology for diagnosis. Urine samples were collected aseptically by cystosynthesis. Clinically suspected specimens of pyometra and prostate infection were collected by steril containers or transport swabs. Swabs samples for bacterial examination were taken from by vaginal way. A total of 110 samples of obtained from cats and dogs were examined. From dogs 33 urine samples (23 from female dog; 10 from male dog), 30 uterus content from pyometra and 1 prostate content; from cats 44 urine (25 from female cat; 19 from male cat) and 2 uterus content from pyometra were examined in terms of E.coli. The samples were brought to laboratory under aseptic and cold chain conditions (+2-+8 °C) and their analyses were performed promptly.

Isolation and identification:

The urine samples were inoculated onto blood agar base (Merck, Germany) with 5% sheep blood and MacConkey Agar (BD Difco, USA) plates. The plates were incubated at 37°C for 24-48 hours. Besides, urine samples were centrifuged at 1000 g for 20 minutes and then the sediment was inoculated onto the same agar plates (27). To determine of the infection level by colony counting method in urine (E. coli and other bacteria), 0.1 ml urine samples were inoculated on Cystine-Lactose-Electrolyte-Deficient Agar (CLED Agar, BD Difco, USA) and nutrient agar and they were incubated at 37°C for 18-24 hours. After the incubation, colonies were evaluated by Litster et al. (22) as very light (100-999 colony forming unit (cfu/ml), light (1000-9999 cfu/ml), moderate (10.000-99.000 cfu/ml) and heavy (≥100.000 cfu/ml). Also, uterus swabs were and prostate content were inoculated onto blood agar base (Merck, Germany) 5% sheep blood and MacConkey Agar (BD Difco, USA) plates and incubated at 37°C for 24-48 hours (15). All colonies were identified with spesific conventional and phenotypic methods for *E. coli*. (29).

Investigation of virulence factors by phenotypic methods:

E. coli isolates were tested for the haemolysis by phenotypically on 5% sheep blood agar and they were incubated at 37°C for 24 hours. Haemolysis was observed after overnight incubation at 37°C (7). In order to do haemagglutination tests, *E. coli* colonies inoculated onto Trypticase soy agar (TSA, Oxoid, England) were subcultured on MacConkey agar and incubated at 37°C for overnight. Then, a single colony from each strain was inoculated into Mueller-Hinton broth (Oxoid, England) and was incubated for 5 days at 37°C for enrichment of fimbriae. At the end of incubation, isolates were subcultured to Casamino acid Yeast Extract Agar and incubated at 37°C for overnight. Pure erythrocytes were obtained from sheep and chicken blood with anticoagulant. Erythrocyte suspensions were prepared with and without 3% D-mannose with PBS. Each *E.coli* strain was suspended in PBS and were mixed with red blood cell suspensions (1). The results were evaluated in terms of mannose-resistant haemagglutination (MRHA) and mannose-sensitive haemagglutination (MSHA) (11).

M9 broth and M9 agar (both are containing 0.2 mM 2,2 '-dipyridil) were prepared and used for the presence of aerobactin production phenotypically. E. coli isolates were activated on TSA and inoculated into M9 broth and they were incubated at 37°C for 24 hours. After the incubation, broth cultures of 1mL were transferred to M9 broth and

incubated at 37°C for 24 hours. Then, cultures were streaked on M9 Agar and incubated 37°C for 48 hours. After the incubation period, bacterial growth was evaluated as positive for aerobactin production (9, 35).

For identifing of sera resistance phenotypically, *E. coli* isolates were activated on TSA and diluted to Mc Farland No:3 with PBS. Then, 0.25 mL of this suspension was mixed with 5 mL of TSA agar at 50°C, and poured into the petri dishes. Cattle and sheep sera were inserted to the small pits in the petri dishes, stored at 4°C for 4 hours, and then incubated at 37°C for overnight. The test was evaluated in terms of the presence of the growth around the wells (33).

Polymerase chain reaction (PCR) detection of virulence genes:

E. coli isolates were inoculated in Luria Bertani Broth (LB, BD Difco, USA) incubated at 37°C for 18 hours. Following the incubation, 1 mL LB broth tubes containing isolates were centrifuged at 5000 g for 10 min. At the end of centrifugation, supernatant was removed and the pellet was used for DNA extraction. Fermentas GeneJET Genomic DNA Purification kit (Fermentas, Lithuanian) DNA Purification Protocol for Gram negative bacteria were used for DNA extraction.

hlyA (Haemolysin) , *fimH* (Type-1 fimbria), *papC* (P fimbria), *iucD* (aerobactin), *cnf1* (cytotoxic necrotizing factor), *traT* (serum resistance), *sfaDE* (S fimbria), *afaBC* (afimbrial adhesin), *cvaC* (colisin) and *usp* (uropathogenic spesific protein) genes were investigated by PCR using commercially synthesized specific primer sets. The base sequences of the primers, corresponding to gene regions, product lengths, and references have been shown in Table 1.

Table 1: Primer sequences, Target genes, base pairs and references

Tablo 1: *Primer sekansları, hedef genler, baz büyüklükleri ve referanslar*

Primers	Sequence	Target Gene	Base Pairs	References
hly1	5'-aacaaggataagcactgttctggct-3'	*hlyA*	1177 bp	Yamamoto et. al. (41)
hly2	5'-accatataagcggtcattcccgtca-3'			
fimH- F	5'-tcgagaacggataagccgtgg-3'	*fimH*	508 bp	Johnson and Stell (18)
fim H- R	5'-gcagtcacctgccctccggta-3'			
pap1	5'-gacggctgtactgcagggtgtggcg-3'	*papC*	328 bp	Yamamoto et. al. (41)
pap2	5'-atatcctttctgcagggatgcaata-3'			
aer1	5'-taccggattgtcatatgcagaccgt-3'	*iucD*	602 bp	Yamamoto et. al. (41)
aer2	5'-aatatcttcctccagtccggagag-3'			
tratF	5'-ggtgtggtgcgatgagcacag-3'	*traT*	290 bp	Johnson and Stell (18)
tratR	5'-cacggttcagccatccctgag-3'			
sfa1	5'-ctccggagaactgggtgcatcttac-3'	*sfaDE*	410 bp	Yamamoto et. al. (41)
sfa2	5'-cggaggagtaattacaaacctggca-3'			
afa1	5'-gctgggcagcaaactgataactctc-3'	*afaBC*	750 bp	Yamamoto et. al. (41)
afa2	5'-catcaagctgtttgttcgtccgccg-3'			
uspF	5'- acattcacggcaagcctcag-3'	*usp*	440 bp	Bauer et al. (5)
uspR	5'-agcgagttcctggtgaaagc-3'			
cnf1	5'-aagatggagtttcctatgcaggag-3'	*cnf1*	498 bp	Yamamoto et. al. (41)
cnf2	5'-cattcagagtcctgccctcattatt-3'			
cvaCF	5'-cacacacaaacgggagctgtt-3'	*cvaC*	679 bp	Johnson and Stell (18)
cvaCR	5'-cttcccgcagcatagttccat-3'			

Protocol and amplification conditions, which were described by Yamamoto et al. (41) were modified and optimised to work with single PCR. Because of optimisation tests of reference strains was worked well, the same amplification conditions and reaction mix used for all genes. The PCR reaction mix for all genes was performed in a total volume of 25 µl, containing 2 µl of DNA extract (template DNA) and 23 µl of PCR mix. PCR mix consisted of 2.5 µl 10xPCR Buffer (Fermentas, Lithuanian), 0.5 µl of 10mM dNTP (Fermentas, Lithuanian), 0.1 µl of Primer I (Operon, Germany), 0.1 µl of Primer II (Operon, Germany), 2 µl of magnesium chloride (MgCl2) (Fermentas, Lithuanian), 0.2 µl of Taq DNA Polymerase (Fermentas, Lithuanian) and 17.6 µl of DEPC water (Fermentas, Lithuanian). Amplification conditions for PCR was 1 cycle of pre-denaturation at 94 °C (3 minutes), 30 cycles of denaturation at 94 °C (1 minute), annealing at 63 °C (1 minute), extension at 72 °C (2 minutes), 1 cycle of the final extension at 72 °C (7 minutes).

All PCR amplicons (10 µl ampicon and 2 µl 6xLoading Dye- Fermentas, Lithuanian) were electrophoresed at 180 V for one hour on 1.5% agarose gel and visualised on a bio- visualising system (Gene Genius, Syngene). DNA molecular weight marker (Gene Ruler 100bp DNA Ladder plus, Fermentas, Lithuanian) was used.

Reference *E. coli* strains were used in conventional and PCR tests. These strains were provided by Prof. Dr. James R. Johnson, University of Minnesota, Department of Medicine, Infectious Diseases Fellowship Programme Director. These reference strains codes are J96, L31, 2H25, V27, PM9, 2H16.

3. Results

A total of 45 *E. coli* were isolated. These isolates isolated from 23 (69.7%) of 33 urine specimens, 15 (50%) of 30 uterus contents from pyometra infections, and 1 (100%) of a prostate content from dogs. 5 (11.3%) of 44 urine specimens and 1 (50%) of 2 uterus contents from pyometra infections from cats.

According to the results of colony counting for infection level in urine, 23 urines of dogs were found to be heavy infected. 1 of dog urine samples was moderate and 9 of dogs urine samples were found to be very light infected. *E.coli* was isolated from all of the urines of 23 dogs which were heavy infected. While 19 of cat urines were found to be heavy infected, *E. coli* was isolated from 5 of these urine samples. 25 of cat urines were found to be very light infected.

In haemolysis analysis of *E. coli* isolates; 33 (73.3%) of all isolates including 18 isolated from dog urine cultures, 12 from pyometra infections of dogs, 1 isolated from prostate content, 1 isolated from cat urine, and 1 isolated from pyometra infections of cats had haemolytic activity on blood agar. While 44 (97.7%) *E. coli* isolates were haemagglutination positive with sheep erythrocytes, 40 (88.8%) *E. coli* isolates were positive with chicken erythrocytes. All haemagglutination positive strains were identified as MRHA.

From all *E.coli* isolates, 9 (20%) were positive for aerobactin production. While all isolates showed sensitivity to sheep sera, 26.7% of the isolates were resistant to bovine sera.

In PCR examinations of 45 *E.coli*, hlyA gene was detected in 73.3% of all isolates. fimH was found in 100%, papC in 71.1%, sfaDE in 82.2%, and afaBC in 0% of the isolates. iucD, traT, usp, cnf1 and cvaC genes were found in 20%, 71.1%, 84.4%, 75.5%, and 11.1% of the isolates, respectively. The results of virulens genes of *E. coli* strains isolated from urine, uterus and prostate contents of cats and dogs have been shown in Table 2 and Figures 1 and 2.

Table 2: PCR results of virulence genes

Tablo 2: *Virulens genlerine ait PCR sonuçları*

Animal Species	***E.coli* Isolates (n) (%)**	***hlyA***	***fim*H**	***pap*C**	***iucD***	***tra*T**	***cnf*1**	***sfa*DE**	***afa*BC**	***cvaC***	***usp***
	Urine	18	23	17	4	17	18	19	0	5	19
	n:23	(78.2)	(100)	(73.9)	(17.3)	(73.9)	(78.2)	(82.6)	(0)	(21.7)	(82.6)
Dog	Uterus Content	12	15	11	2	10	13	13	0	0	14
	n:15	(80)	(100)	(73.3)	(13.)	(66.6)	(86.6)	(86.6)	(0)	(0)	(93.3)
	Prostate Content	1	1	1	0	1	1	1	0	0	1
	n:1	(100)	(100)	(100)	(0)	(100)	(100)	(100)	(0)	(0)	(100)
	Urine	1	5	2	3	4	1	3	0	0	3
	n:5	(20)	(100)	(40)	(60)	(80)	(20)	(60)	(0)	(0)	(60)
Cat	Uterus Content	1	1	1	0	1	1	1	0	0	1
	n:1	(100)	(100)	(100)	(0)	(100)	(100)	(100)	(0)	(0)	(100)
	n: 45	33	45	32	9	32	34	37	0	5	38
	(100)	(73.3)	(100)	(71.1)	(20)	(71.1)	(75.5)	(82.2)	(0)	(11.1)	(84.4)

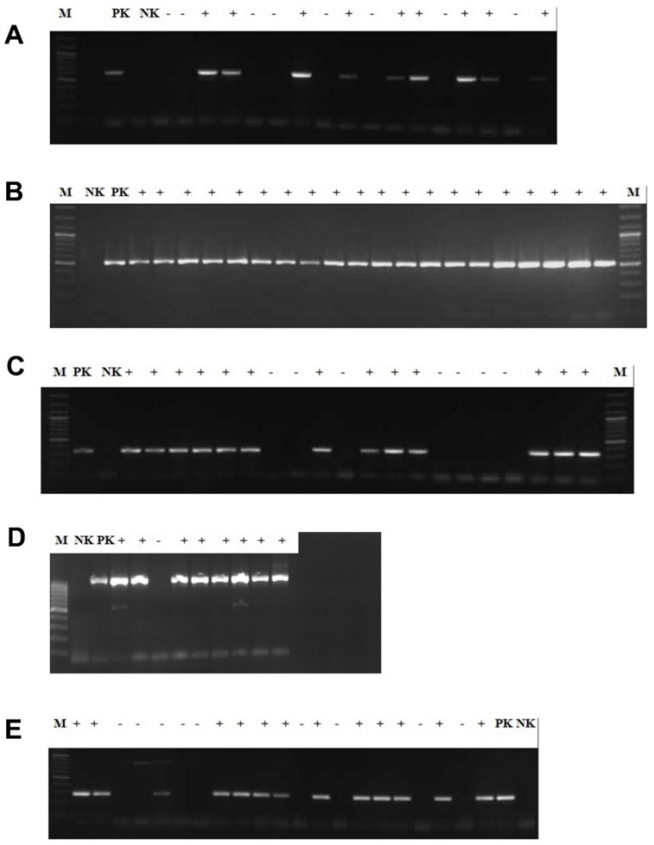

Figure 1: (a) *iucD* gene, 602bp, **(b)** *fimH* gene, 508bp, **(c)** *papC* gene, 328bp, **(d)** *hlyA* gene, 1177bp, **(e)** *traT* gene, 290bp

Şekil 1: (a) *iucD geni, 602bp,* ***(b)*** *fimH geni, 508bp,* ***(c)*** *papC geni, 328bp,* ***(d)*** *hlyA geni, 1177bp,* ***(e)*** *traT geni, 290bp*

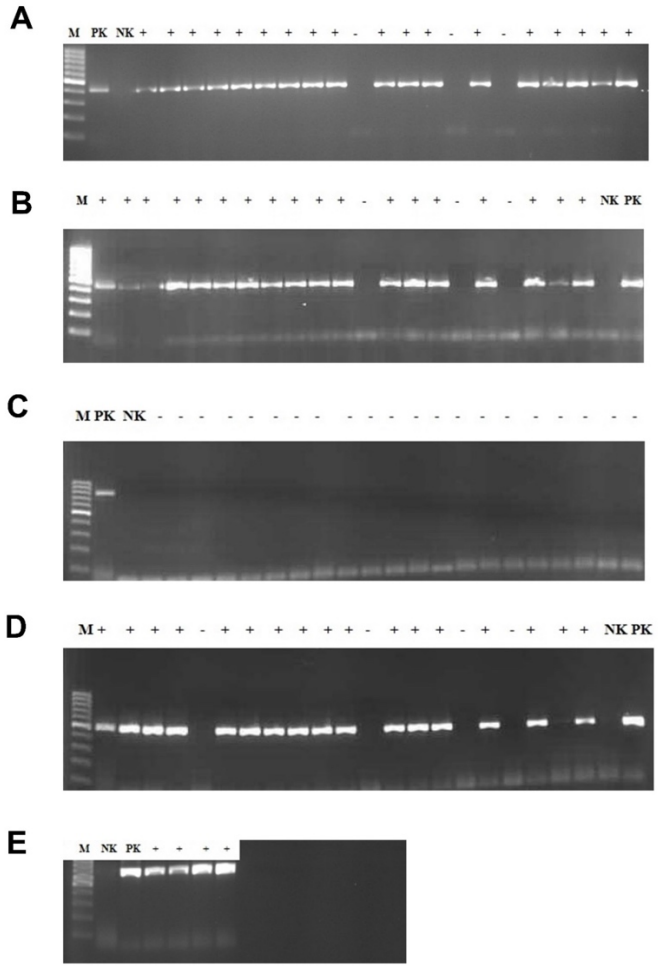

Figure 2: (a) *sfaDE* gene, 410bp, **(b)** *usp* gene, 440bp, **(c)** *afaBC* gene, 750bp**, (d)** *cnf1* gene, 498bp, **(e)** *cvaC* gene, 679bp
Şekil 2*: (a) sfaDE geni, 410bp, (b) usp geni, 440bp, (c) afaBC geni, 750bp, (d) cnf1 geni, 498bp, (e) cvaC geni, 679bp*

4. Discussion and Conclusion

Virulence factors of *E. coli* which were isolated from urinary tract infections and pyometra from dogs and cats very limited studied and reported in Turkey and world.

Gatoria et al. (13) detected 100.000 and above cfu/ml in 7 of 21, 1.000 and above cfu/ml in 3 of 21, and 100 and above cfu/ml in 1 of 21 urine samples. Also, they could not detect any bacterial growth in 9 urine samples. Litster et al. (22) examined urine samples, from which 44 bacteria were isolated, and they found 100 000 and above cfu/ml in 39 of 44 isolates and 10.000-99.999 cfu/ml 5 of 44 isolates. In this study, while 19 of cat urines were found to be heavy infected but, *E. coli* was isolated from 5 of these urine samples. Otherwise, *E. coli* was isolated from all urine of dogs (n:23), which were found over 100.000 cfu/ml of bacteria. Compared with other studies results of the infection level and isolation rate of *E.coli* from urine samples of cats and dogs, only *E. coli* can cause heavy infection in dogs urinary system. These results had importance considering clinical microbiology especially for dogs.

One of the virulence factors, α-haemolysin (*hlyA*), is an important virulence both intestinal and extra intestinal infections in *E. coli* strains. (7). There is a limited number of studies on determination of haemolytic activity of strains isolated from the urogenital tract infections in dogs and cats. Hemolysine is secreted by the majority of pathogenic *E. coli* strains. (16). In particular, the importance of α-hemolysis is due to the fact that it is strongly inflamed, which leads to the secretion of IL-6 and chemotaxin in the pathogenesis of kidney disease. Clinical studies have demonstrated that

the virulence factors of *E. coli*-like hemolysis production and the capacity of the serum to counteract the antiseptic effect play a role in pathogenesis. (26). In this study, a high level of α-haemolysin was detected in *E.coli* strains But in *E.coli* strains isolated from dog urine, α-hemolysin was found to be higher than cat urine isolates. This could be associated with number of isolated strains and infection fo the level in urine. According to the isolation rate of *hlyA* and haemolytic activity, this virulence factor is commonly seen in these strains and important in these infections especially pathogenesis.

MRHA and haemolysin synthesis are common especially among the *E.coli* strains. MRHA and haemolytic *E. coli* strains release more histamine from the mast cells only than haemolytic *E. coli* strains. This view may indicate a possible synergistic effect between mannose resistance and other virulence factors in uropathogenic *E.coli* strains (19). Compaired MRHA and rate of haemolytic strains in this study, it is supported to Johnson (19) who signified possible synergistic effect between mannose resistance and other virulence factors in uropathogenic *E.coli* strains.

Some studies have reported that no sfa and afa gene were detected in *E. coli* isolated from poultry. (8). In this study, since genes encoding these fimbriae were detected at high rates genotypically; our study results showed that Type 1, S and P fimbriae played an important role in invasions, spread and pathogenesis of urinary tract and genital system infections.

In this study, all *E.coli* strains were genotypically positive for *fimH* gene. On this subject, in some studies it was reported that expression of fimbriae was controlled by type 1 fimbriae operon. Some fimbriated *E. coli* isolates may change their genotype in passage from fimbriated to afimbriated state and it is reported that the use of various methods may be useful based on this change in order to increase the synthesis of fimbriae. However, it has not been completely successful (3,14). There are methods to increase the synthesis of fimbriae (14). In this study, E. coli strains were incubated at 37°C for 5 days for the enrichment of fimbriae and at the end of this period, MSHA was not found in dogs and cats *E. coli* isolates. This situation supported Arthur et al. (3) and Gürdal (14) and showed that the results could be different between fimH gene and phenotype.

Against the lethal effect of serum on bacteria, resistance could be developed by bacterial capsular polysaccharides, 'O' polysaccharides, and surface proteins (19). Serum resistance was investigated in *E.coli* strains from infections seen in several animal species. Our results showed that serum resistance and sensitivities could be different in *E. coli* strains isolated from different animal species. According to these results, it was thougth that the use of the serum of other animal species will be increases the knowledge in phenotypic methods. In addition, this may be due to study of different gene regions responsible for serum resistance or regional differences between studies.

Cytotoxic necrotizing factor plays a role in the pathogenesis of urogenital system infections and is an important virulence factor because of causing tissue damage (8,16). *cnf1* gene was detected in (75.5%) of all strains in this study. Wells et. al. (39) reported that they detected 25% cnf1 gene in 159 E. coli isolates from canine patients in 2007. This result showed that cytotoxic necrotizing factor was common and important virulence factor in uropathogenic *E. coli* and prognosis of infection.

Colicin V gene encoding *cvaC* was not detected in *E.coli* strains isolated from cats by PCR. This may be associated with the number of isolations.

usp gene was determined in uropathogenic *E.coli* (UPEC) strains in the studies by Kurazono et al. (21), and Yamamoto et al. (40), reporting that the virulence factor encoded by this gene increased infectivity of *E.coli* strains. In our study, Isolates was found to be a high rate positive for *usp* gene. This result suggested that this strain may have higher infectivity.

Evaluation of the isolation and virulence gene incidence of *E. coli* strains in dogs and cats from urogenital system infections, it could be concluded that *E.coli* is an important agent especially in dogs. *E. coli* was isolated from all urine of dogs over 100.000 cfu/ml of bacteria. This result was found to be significant in terms of aetiopathogenesis of infection and clinical microbiology. However, it was thought that urine and uterine samples of healthy cats and dogs should be examined for *E. coli* and virulence factors in other studies.

Haemolysis, type 1 fimbriae, P fimbriae, S fimbriae, cnf, serum resistance, uropathogenic specific protein were detected at high rates by molecular method. Because of that, it is concluded these virulence factors are important for the pathogenesis of *E. coli* infections in urinary tract and genital system infections. Aerobactin production and

haemolysis were determined at the same rate by using conventional and molecular methods. However, for serum resistance, phenotypic findings were higher than genotypic results. This result revealed the effectiveness of the management of molecular analysis of virulence properties. And also it should be considered that the serum resistance associated with gene *traT* may not be expressed in vitro.

According to this study, in *E. coli* strains isolated from dogs, virulence factors were found to be higher than cat isolates (Table 2). However, colisin encoding gene (*cvaC*) only were detected from 5 of urine samples from dogs. This virulence factor could be important for the urinary tract infections in dogs rather than cats. Therefore, it is concluded that it would be useful to routine diagnosis using both conventional and molecular methods to investigate the virulence factors in clinical microbiology.

According to these study results, conducting more comprehensive studies on determination of virulence properties of *E. coli* strains isolated from urogenital tract infections and investigation of other genes encoding virulence factors would increase the knowledge on aetiopathogenesis of the disease and this knowledge will be increase the success of the treatment.

Conflict of Interest

The author declared no conflict of interest.

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Authors' Contributions

Idea / concept: Orkun Babacan and Müjgan İzgür
Experiment design: Orkun Babacan
Supervision / Consultancy: Müjgan İzgür
Data collecting: Orkun Babacan
Data analysis and interpretation: Orkun Babacan
Literature search: Orkun Babacan
Writing the article: Orkun Babacan
Critical review: Orkun Babacan and Müjgan İzgür

Ethical Approval

An ethical statement was received from the authors that the data, information and documents presented in this article were obtained within the framework of academic and ethical rules, and that all information, documents, evaluations and results were presented in accordance with scientific ethics and moral rules.

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