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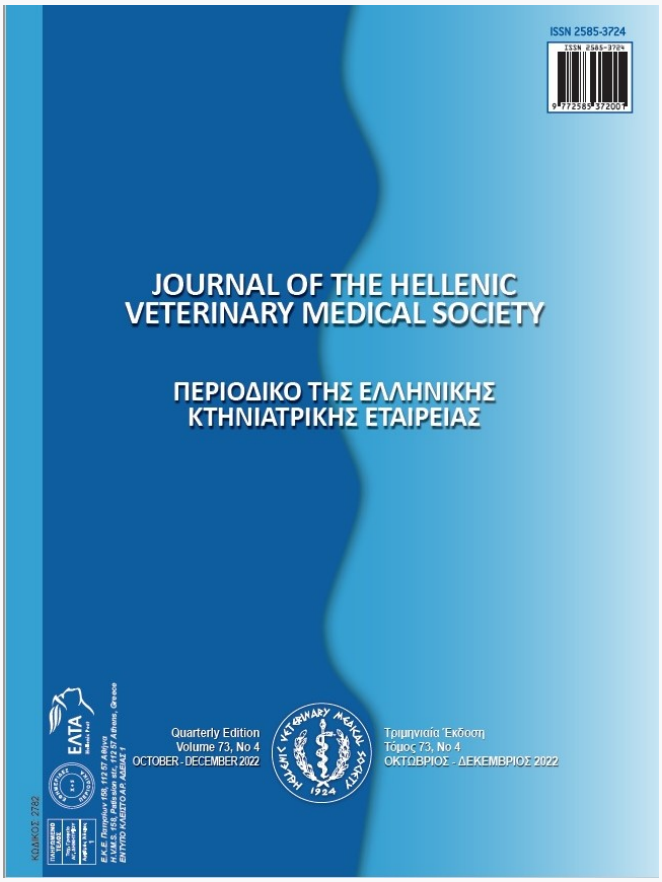

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Mobile resistance determinants, plasmid replicon types and phylogeny among *Escherichia coli* strains isolated from cats and dogs

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ABSTRACT: Multidrug resistance is a great challenge for the treatment of infectious diseases. We determined antibiotic resistance patterns, integrons, plasmid-mediated ESBL-, AmpC beta-lactamase-, carbapenemase-, colistin resistance genes, plasmid replicon types and phylogeny of fecal *Escherichia coli* strains isolated from domestic cats and dogs in Turkey.

A total of 104 fecal samples of healthy 49 cats and 55 dogs were examined. The integrons, plasmid-mediated resistance genes, plasmid replicon types and phylogroups were determined by PCR. Antimicrobial susceptibilities were performed by disc diffusion and microdilution methods.

Escherichia coli strains were mostly resistant to AMP (56.73%), SXT (39.42%), CTX (38.46%) and CIP (30.77%). Colistin resistance was not detected. ESBL and carbapenemase rates were 35.5 % and 7.69%, respectively. Eighty (76.9%) and 49 (47.1%) strains were harboring class I and class II integrons, respectively. Besides 12 strains were shown to possess class III integrons. The most frequently detected genes were bla_{CTX-M} (48.08%), bla_{TEM}(45.19%) and bla_{VIM}(20.19%). In our study, none of strains were positive for *mcr-1* and *mcr-2* genes. Integrons were mostly found on plasmids of incompatibility groups IncF (71.25%) and strains bearing bla_{CTX-M} and bla_{TEM} carried a wide range of plasmid replicons of which IncF, IncFIB, IncK, and IncN. The majority of the strains were grouped in B2 (31.73%) and B1 (22.12%) and resistant bacteria mostly belonged to phylogroup B2.

We showed an increasing trend in ESBL-producing *E. coli* among fecal microbiota members. *E. coli* strains with different plasmid replicon types and phylogroups isolated from cats and dogs can be resistant to various antibiotics which are used in human and veterinary medicine.

Key-words: *E.coli;*integrons;plasmid–mediated resistance genes;plasmid types; phylogeny

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INTRODUCTION

Emergence, dissemination and selection of bacteria with acquired resistance mechanisms continue to complicate the management of infectious diseases worldwide. It is important to note that the resistance genes are spreading among humans, animals, even plants, and the environment via mobile genetic elements such as plasmids, transposons, and integrons (Dolejska and Papagiannitsis, 2018; Van Puyvelde et al., 2018; Hernando-Amado et al., 2019). Pathogens are not the only ones carrying antibiotic resistance genes; environmental bacteria and microbiota bacteria of humans, animals and plants can also harbor antibiotic resistance genes; and spreading of resistant bacteria within and between these sectors become very important in domestic animals which play a reservoir role for antibiotic-resistant bacteria due to their close relations to humans. In this sense, One Health is a very important collaborative initiative of multiple science professions to achieve optimal health for people, animals, plants, and their environment (Sunde et al., 2015; Taggar et al., 2020).

Previous investigations focused on integrons which are enabled to capture, integrate and express gene cassettes and lead to play an important role in the dissemination of multiple antibiotic resistances within microbial populations(Carattoli et al., 2006; Cocchi et al., 2007; Madec et al., 2017; Weiss et al., 2018; Dolejska and Papagiannitsis, 2018; Taggar et al., 2020). On the other hand, the spread of plasmid-mediated resistance genes, ESBL, pAmpC, carbapenemase and colistin resistance genes becomes important in human and veterinary medicine worldwide (Sunde et al., 2015; Madec et al., 2017; Dolejska and Papagiannitsis, 2018; Pulss et al., 2018; Chen et al., 2019; Hagel et al., 2019; Taggar et al., 2020; Bandyopadhyay et al., 2021; Lei et al., 2021; Shin et al., 2021). Epidemiological studies determining plasmid replicon types and phylogroups of *Escherichia coli* strains gain importance (Carattoli et al., 2006; Clermont et al., 2013; Jackson et al., 2015; Rocha-Gracia et al., 2015; Bourne et al., 2019; Abreu-Salinas et al., 2020).

This study was designed to investigate the antibiotic resistance, integrons, plasmid-mediated ESBL/AmpC beta-lactamase,carbapenemase and colistin resistance genes, plasmid replicon types and phylogeny of *Escherichia coli*strains isolated from domestic cats and dogs.

MATERIALS AND METHODS

Sample collection, isolation and identification of *Escherichia coli* strains

In the present study, a total of 104 fecal samples of pets (49 cats and 55 dogs) obtained from individually owned animals in Istanbul, during their routine examination at three veterinary clinics where animals were admitted for routine physical examination, parasite screening, vaccination and grooming or directly by their owners. Pet fecal samples were collected from the ground or litter box by their owners and veterinarians.There was no invasive procedure for collecting fecal samples.

Fresh fecal samples were transferred via a sterile container. The samples were cultured on Mac Conkey and Chromogenic media; then incubated for 24 hours at 37˚C. Colonies with typical *E. coli* morphology were further identified by conventional biochemical testing. The isolates were stored in tryptic soy broth containing 20% glycerol at -80°C for further analysis. This study was performed from June 2019 to November 2020.

Determination of antibiotic susceptibilities, ESBL and carbapenemase production

We performed the Kirby-Bauer disc diffusion method for 17 different antimicrobial agents except colistin: ampicillin (AMP) (10 ug), cefotaxime (5 ug) (CTX), ceftazidime (5 ug) (CAZ), cefepime (30 ug) (FEP), cefoxitin (30 ug) (FOX), amoxicillin/clavulanate (AMC) (20/10 ug), piperacillin-tazobactam (30/6 ug) (TZP), imipenem (10 ug) (IMP), meropenem (10 ug) (MER), ertapenem (10 ug) (ERT), amikacin (10 ug) (AK), levofloxacin (5 ug) (LEV), ciprofloxacin (5 ug) (CIP), chloramphenicol (30 ug) (C), gentamicin (10 ug) (GN), trimethoprim-sulfamethoxazole (1.25/23.75 ug) (SXT) and colistin (COL) (10 ug). The results were interpreted as recommended by EUCAST Guidelines (EUCAST, 2020).

The double-disc synergy test was performed with cefotaxime, ceftazidime and cefepime in the proximity of amoxicillin-clavulanic acid for the screening of ESBL (CLSI, 2021).

Carbapenem-resistant strains were examined for susceptibility to colistin (COL) by microdilution method with a commercial kit (Diagnostics sru, Slovakia) according to the guidelines of the CLSI(CLSI, 2021). We used *E. coli* ATCC 25922 as quality control.

Determination of integrons, plasmid-mediated resistance genes, plasmid replicon types and phylogroups

The integrons, plasmid-mediated resistance genes, plasmid replicon types and phylogeny were determined by PCR using suitable primers (Table 1).

Isolation of genomic and plasmid DNA

Genomic and plasmid DNAs were extracted from overnight cultures of *E. coli* strains grown in tryptic soy broth (TSB) at 37°C by a commercial kit (GeneDireX, Taiwan) according to the manufacturer's guidelines.

Detection of class I-II-III integron-related genes and plasmid-mediated ESBL/pAmpC, carbapenemase and colistin resistance genes

For the detection of integron genes (*intI*, *intII, intIII),*both genomic and plasmid DNAs, for ESBL encoding genes (*TEM, SHV, OXA* and *CTX-M*), plasmid-mediated ampC beta-lactamase genes (*FOX, CIT, DHA, EBC, MOX* and *ACC*), carbapenemase encoding genes (*KPC, NDM, OXA-48, IMP* and *VIM*) and plasmid-mediated colistin resistance genes (*mcr-1* and *mcr-2*) plasmidic DNAs were used. All analyses were performed by the multiplex PCR method. All used primers were shown in supplementary Table (Goldstein et al., 2001; Pérez-Pérez and Hanson, 2002; Woodford et al., 2006; Fang et al., 2008; Ren et al., 2013; Zowawi et al., 2014; Liu et al., 2016; Xavier et al., 2016; Rebelo et al., 2018). We used a commercial master mix kit (Genemark, Taiwan) for PCR assays. We prepared mixtures (25 μL last volume) according to the manufacturer's instructions (Genemark, Taiwan): 5 μL master mix, 2 μL DNA, 2 μL each primer (1 μL for each primer from 10 pmol concentration) and nuclease-free water.

Multiplex PCRs were performed according to Ren et al., (2013) for detection of integron genes; the reaction conditions for PCR amplification were as follows: initial denaturation for 4 min at 94^0C; degradation for 45 sec at 94°C; annealing for 45 sec at 57°C, elongation for 55 sec at 72°C, final elongation for 4 min at 72°C. These reactions were carried out for 30 cycles (Prima Trio high media thermal cycler, Mumbai, India).

Table 1. Primers used for multiplex PCR for detection of integrons, and antibiotic resistance genes

Target	Forward	Revers
Class I int	CCTCCCGCACGATGATC	TCCACGCATCGTCAGGC
Class II int	GTAGCAAACGAGTGACGAAATG	CACGGATATGCGACAAAAAGGT
Class III int	GCCTCCGGCAGCGACTTTCAG	ACGGATCTGCCAAACCTGACT
CTX-M	ATGTGCAGYACCAGTAARGTKATGGC	TGGGTRAARTARGTSACCAGAAYCAGCGG
TEM	CGCCGCATACACTATTCTCAGAATGA	ACGCTCACCGGCTCCAGATTTAT
SHV	CTTTATCGGCCCTCACTCAA	AGGTGCTCATCATGGGAAAG
OXA	ACACAATACATATCAACTTCGC	AGTGTGTTTAGAATGGTGATC
CTX-M group 1	AAAAATCACTGCGCCAGTTC	AGCTTATTCATCGCCACGTT
CTX-M group 2	CGACGCTACCCCTGCTATT	CCAGCGTCAGATTTTTCAGG
CTX-M group 8	TCGCGTTAAGCGGATGATGC	AACCCACGATGTGGGTAGC
CTX-M group 9	CAAAGAGAGTGCAACGGATG	ATTGGAAAGCGTTCATCACC
CTX-M group 25	GCACGATGACATTCGGG	AACCCACGATGTGGGTAGC
MOX	GCTGCTCAAGGAGCACAGGAT	CACATTGACATAGGTGTGGTGC
FOX	AACATGGGGTATCAGGGAGATG	CAAAGCGCGTAACCGGATTGG
CIT	TGGCCAGAACTGACAGGCAAA	TTTCTCCTGAACGTGGCTGGC
DHA	AACTTTCACAGGTGTGCTGGGT	CCGTACGCATACTGGCTTTGC
ACC	AACAGCCTCAGCAGCCGGTTA	TTCGCCGCAATCATCCCTAGC
KPC	ATCTGACAACAGGCATGACG	GACGGCCAACACAATAGGTG
NDM	GCAGGTTGATCTCCTGCTTG	ACGGTTTGGCGATCTGGT
OXA48	GCGTGGTTAAGGATGAACAC	CATCAAGTTCAACCCAACCG
IMP	CTACCGCAGCAGAGTCTTTGC	GAACAACCAGTTTTGCCTTACC
VIM	GATGGTGTTTGGTCGCATA	CGAATGCGCAGCACCAG
mcr-1	CGGTCAGTCCGTTTGTTC	CTTGGTCGGTCTGTAGGG
mcr-2	TGTTGCTTGTGCCGATTGGA	AGATGGTATTGTTGGTTGCTG

For the detection of ESBL genes, we followed multiplex PCR conditions as suggested by Fang et al., (2008). The cycling parameters were as follows: initial denaturation at 95°C for 15 min, followed by 30 cycles of 94°C for 30 s, 62°C for 90 s, and 72°C for 60 s and final extension at 72°C for 10 min.

For the identification of CTX-M groups (CTX-M group 1, CTX-M group 2, CTX-M group 9, CTX-M group 8, and CTX-M group 25) of strains harboring CTX-M genes; we performed the following PCR conditions: initial denaturation at 94˚C for 5 min, 30 cycles of 94˚C for 25 s, 52˚C for 40 s and 72˚C for 50 s and final elongation at 72˚C for 6 min (Woodford et al., 2006).

The detection of pAmpC beta-lactamase genes, following conditions, were performed: initial denaturation step at 94°C for 3 min, followed by 25 cycles of DNA denaturation at 94°C for 30s, annealing at 64°C for 30s, and extension at 72°C for 1 min. Then, the final extension step at 72°C for 7 min was added as described byPérez-Pérez and Hanson (2002).

Carbapenem resistance genes were determined by PCR conditions described by Zowawi et al., (2014): initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 45 s, extension at 70°C for 60 s, and final extension at 70°C for 5 min.

To investigate the presence of *mcr-1* and *mcr-2* genes, we applied the following conditions: 1 cycle of denaturation at 94°C for 15 min, followed by 25 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 90 s and elongation at 72°C for 60 s, and final cycle of elongation at 72°C for 10 min (Rebelo et al., 2018).

Detection of plasmid replicon types

Plasmid Inc/Rep typing was determined by multiplex and simple PCR methods for 18 different genes including FIA, FIB, FIC, HI1, HI2, I1-Iγ, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FIIA as previously reported by Carattoli et al., (2005a).

Detection of phylogroups

We detected phylogroups of *E.coli* strains using two sequential PCR assays as described by Clermont et al., 2013. Firstly, phylogroups were evaluated according to the presence/absence of TspE4.C2, *chuA*, *yjaA* and *arpA* genes. Then, using *trpAgpC*, *ArpAgpE,* and *trpBA* primers, another multiplex PCR assay was conducted to distinguish the phylogroup D from E, E from *Escherichia* clade I, and A from C.

We used a commercial master mix kit (Genemark, Taiwan) for PCR and mixtures were prepared as mentioned above.

Running conditions were: initial denaturation for 4 min at 94^0C; degradation for 30 s at 94°C; annealing for 30 s at 56°C; elongation for 45 s at 72°C; final elongation for 5 min at 72°C. These reactions were carried out for 30 cycles (Prima Trio high media thermal cycler, Mumbai, India) (Clermont et al., 2013).

Electrophoresis

Amplified DNA products were separated by gel electrophoresis in 1.5% agarose gel by staining in ethidium-bromide (0.5 μg/mL). Then, products were electrophoresed for 40 min under 80 volts with 1XTBE electrophoretic liquid. The visualization of amplicons was provided under UV light using Hi-UV MAX transilluminator and recorded (HiMedia, India). PCR amplicon sizes were compared to DNA ladder (Genemark, Taiwan) which labeled between 100 - 1000 bp.

Statistical analysis

The determination of all relationships was statistically analyzed. The categorical variables were reported as n (%) by using Pearson Chi-Square test, and Fisher's exact test. SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp.) was used for statistical analysis, and a p-value <0.05 was considered as statistically significant.

RESULTS

Antibiotic susceptibilities of the strains

It was found that the highest resistance rates were against AMP (56.73%) followed by CTX (38.46%), CIP (30.77%), FEP (29.81%) and LEV (27.88%) in 104 *E. coli* strains. The most effective antibiotics were amikacin and colistin. As we compared antibiotic resistance rates of the strains isolated from cats and dogs, there was no statistically significant difference ($p > 0.05$).

In our study, 37/104 (35.5%) were found to be ESBL-positive. 19 (51.3%) ESBL positive strains were isolated from dogs. Eight (7.69%) (cat= 4 strains; dog= 4 strains) out of 104 strains were found to be carbapenemase positive.

Resistance rates for CIP, SXT, LEV, AMP, CTX and FEP were found to be statistically significantly higher in strains isolated from animals which are known to be treated with antibiotics in the past ($p<0.001$, $p<0.001$, $p<0.001$, $p=0.004$, $p=0.005$, $p=0.002$, respectively).

The prevalence of integrons and resistance genes

Among 104 *E. coli* strains, 80 (76.9%) and 49 (47.1%) were found to harbor class I and class II integrons, respectively. *intI* gene was detected to be carried in 61 (58.65%) strains on genomic DNA and in 78 (75%) strains on plasmid DNA. Similarly, 34 (32.69%) and 31 (29.81%) of *E. coli* strains harbor *intII genes* on genomic and plasmid DNA, respectively. We found that only 12 strains were shown to carry *intIII* genes which 8 strains encoded on genomic DNA and 7 strains encoded on plasmids; 12 *E. coli* strains were found to possess all three classes of integrons. In total, dogs were significantly found to be more prevalent carriers for strains harboring integrons ($p=0.023$).

Statistical analysis showed a significant correlation between the presence of plasmidic *intI*, *intII* and *intIII* genes and resistance to CTX ($p=0.017$) in cat isolates. Furthermore, cat strains encoding genomic integrons were found to be statistically significantly more resistant to CIP ($p=0.003$), STX ($p=0.001$), AMP ($p=0.013$), CAZ ($p=0.003$), CTX ($p=0.003$) and FEP ($p=0.008$). The presence of class I-II-III integrons in plasmid and genomic DNAs of *E. coli* strains provided from dogs was shown to be correlated with resistance to SXT ($p<0.05$) and AMP ($p < 0.05$). The presence of genomic-encoded class I integrons was shown to be statistically significantly related to the presence of CTX-M ($p= 0.002$).

Although in 104 strains the prevalence of ESBL encoding genes CTX-M (48.08%) and TEM (45.19%) were found to be high, there was no statistically significant difference between strains isolated from cats and dogs. 18 of CTX-M positive strains were found to be positive for the CTX-M-I and 3 were positive for the CTX-M-IX. There was no strain harboring other CTX-M-groups. There is a statistically significant relationship between bla_{CTX-M} and bla_{TEM} genes and AMP, CTX, FEP resistance in cat isolates ($p< 0.05$) and AMP and SXT resistance in dog isolates ($p< 0.003$).

On the other hand, the prevalence of bla_{OXA} was found to be low (4.8%). In the comparison of strains isolated from cats and dogs, bla_{OXA} gene was found more prevalent in strains isolated from cats ($p=0.021$, 10.20%). The prevalence of bla_{VIM} gene was higher in strains from dogs (27.27%) than those from cats (12.24%); the difference was statistically significant ($p=0.057$).

It was found that a statistically significant relationship between dogs' isolates harboring bla_{OXA-48} gene and resistance to AMC, CAZ, FOX, CTX and FEP ($p< 0.05$).

In our study, none of strains were found to be positive for *mcr-1* and *mcr-2* genes. The prevalence of all resistance genes and integrons were shown in Table 2.

Table 2. The prevalence of integrons and resistance genes

		Total (n=104)	Cats (n=49)	Dogs (n=55)	p-value
Integron					
(Total)	-	20(19.23%)	14(28.57%)	6(10.91%)	0.023[b]
	+	84(80.77%)	35(71.43%)	49(89.09%)	
Plasmidicintegrons					
Integron I					
	-	26(25%)	14(28.57%)	12(21.82%)	0.427[b]
	+	78(75%)	35(71.43%)	43(78.18%)	
Integron II					
	-	73(70.19%)	40(81.63%)	33(60.00%)	0.016[b]
	+	31(29.81%)	9(18.37%)	22(40.00%)	
Integron III					
	-	97(93.27%)	44(89.80%)	53(96.36%)	0.250[a]
	+	7(6.73%)	5(10.20%)	2(3.64%)	

		Total (n=104)	Cats (n=49)	Dogs (n=55)	p-value
Genomic integrons					
Integron I					
	-	43(41.35%)	27(55.10%)	16(29.09%)	0.007[b]
	+	61(58.65%)	22(44.90%)	39(70.91%)	
Integron II					
	-	70(67.31%)	45(91.84%)	25(45.45%)	<0.001[b]
	+	34(32.69%)	4(8.16%)	30(54.55%)	
Integron III					
	-	96(92.31%)	46(93.88%)	50(90.91%)	0.719[a]
	+	8(7.69%)	3(6.12%)	5(9.09%)	
ESBL genes					
CTX-M					
	-	54(51.92%)	25(51.02%)	29(52.73%)	0.862[b]
	+	50(48.08%)	24(48.98%)	26(47.27%)	
TEM					
	-	57(54.81%)	28(57.14%)	29(52.73%)	0.652[b]
	+	47(45.19%)	21(42.86%)	26(47.27%)	
SHV					
	-	104(100%)	49(100%)	55(100%)	-
	+	0	0	0	
OXA					
	-	99(95.19%)	44(89.80%)	55(100%)	0.021[a]
	+	5(4.81%)	5(10.20%)	0	
pAmpC genes					
MOX					
	-	101(97.12%)	48(97.96%)	53(96.36%)	>0.99[a]
	+	3(2.88%)	1(2.04%)	2(3.64%)	
FOX					
	-	104(100%)	49(100%)	55(100%)	-
	+	0	0	0	
CIT					
	-	104(100%)	49(100%)	55(100%)	-
	+	0	0	0	
DHA					
	-	100(96.15%)	47(95.92%)	53(96.36%)	>0.99[a]
	+	4(3.85%)	2(4.08%)	2(3.64%)	
EBC					
	-	104(100%)	49(100%)	55(100%)	-
	+	0	0	0	
ACC					
	-	104(100%)	49(100%)	55(100%)	-
	+	0	0	0	
Carbapenemases genes					
OXA-48					
	-	97(93.27%)	46(93.88%)	51(92.73%)	>0.99[a]
	+	7(6.73%)	3(6.12%)	4(7.27%)	
NDM					
	-	103(99.04%)	48(97.96%)	55(100%)	0.471[a]
	+	1(0.96%)	1(2.04%)	0	
KPC					
	-	104(100%)	49(100%)	55(100%)	-
	+	0(0%)	0	0	

		Total (n=104)	Cats (n=49)	Dogs (n=55)	p-value
VIM					
	-	83(79.81%)	43(87.76%)	40(72.73%)	0.057[b]
	+	21(20.19%)	6(12.24%)	15(27.27%)	
IMP					
	-	101(97.12%)	49(100%)	52(94.55%)	0.245[a]
	+	3(2.88%)	0	3(5.45%)	

Data are expressed as n(%).
a: Fisher's Exact Test, b:Pearson Chi-Square Test; +: strain carries the gene; -: strain does not carry the gene

Plasmid replicon types

Plasmid typing analyses showed that among 104 strains, 76 (73%) were shown to carry more than one plasmid, some carrying up to five. However, 6 strains (5.76%) were shown to belong to none of the plasmid replicon types which we have investigated. The most common plasmid replicon types were F (67.3%), K (50.96%), FIB (46.15%), N (28.85%) and I1-Iγ (23.08%). Between the prevalencesof plasmid replicon types in strains isolated from cats and dogs, there were no statistically significant differences (p >0.05) (Table 3).

The correlation of F plasmid replicon type to CIP (p=0.013), C (p=0.023), LEV (p=0.011), AMP (p=0.008), AMC (p=0.002), CAZ (p=0.002), FOX (p=0.010), CTX (p=0.009) and FEP (p=0.019) resistance was found to be statistically significant.

Among 37 ESBL-positive strains, F plasmid type (81%) was the most prevalent type which was followed by N (43.2%), F1A (37.8%) and K (35.1%). Their presence was statistically significant (p=0.026, p=0.016, p<0.001 and p=0.016, respectively). Furthermore, F plasmid was the most common plasmid replicon type in carbapenemase-producing seven strains followed by FIA (n=5), FIB (n=4), N (n=4), K (n=3) replicon types.

Strains carrying plasmidic integrons (class I-II-III) and plasmid replicon types were investigated. It was shown that the most prevalent plasmid replicon type was F type (71.25%, n= 57) but there was no statistical significance (p= 0.118); the presence rate of K replicon type (56.25%, n=45) followed it. Besides, there was a significant correlation between the presence of integrons and K-type plasmid (p= 0.049).

There was no statistically significant relationship between the bla_{CTX-M} and bla_{TEM} resistance genes and the most prevalent plasmid replicon types (F, F1B K, and N types). On the other hand, there was a statistically significant relationship between bla_{OXA-48} and N plasmid replicon type (p= 0.002), bla_{VIM} gene and I1-Iγ plasmid replicon type (p=0.007), P (p= 0.025) and B/O (p= 0.039) plasmid replicon type.

Table 3. The prevalence of plasmid replicon types

		Total (n=104)	Cats (n=49)	Dogs (n=55)	p-value
HI1					
	-	104(100%)	49(100%)	55(100%)	-
	+	0	0	0	
HI2					
	-	103(99.04%)	49(100%)	54(98.18%)	>0.99[a]
	+	1(0.96%)	0	1(1.82%)	
I1-Iγ					
	-	80(76.92%)	41(83.67%)	39(70.91%)	0.123[b]
	+	24(23.08%)	8(16.33%)	16(29.09%)	
X					
	-	104(100%)	49(100%)	55(100%)	-
	+	0	0	0	
L/M					
	-	104(100%)	49(100%)	55(100%)	-
	+	0	0	0	

		Total (n=104)	Cats (n=49)	Dogs (n=55)	p-value
N					
	-	74(71.15%)	32(65.31%)	42(76.36%)	0.214[b]
	+	30(28.85%)	17(34.69%)	13(23.64%)	
F1A					
	-	85(81.73%)	37(75.51%)	48(87.27%)	0.121[b]
	+	19(18.27%)	12(24.49%)	7(12.73%)	
F1B					
	-	56(53.85%)	28(57.14%)	28(50.91%)	0.524[b]
	+	48(46.15%)	21(42.86%)	27(49.09%)	
W					
	-	104(100%)	49(100%)	55(100%)	-
	+	0	0	0	
Y					
	-	90(86.54%)	43(87.76%)	47(85.45%)	0.732[b]
	+	14(13.46%)	6(12.24%)	8(14.55%)	
P					
	-	100(96.15%)	46(93.88%)	54(98.18%)	0.341[a]
	+	4(3.85%)	3(6.12%)	1(1.82%)	
FIC					
	-	102(98.08%)	49(100%)	53(96.36%)	0.497[a]
	+	2(1.92%)	0	2(3.64%)	
A/C					
	-	103(99.04%)	49(100%)	54(98.18%)	>0.99[a]
	+	1(0.96%)	0	1(1.82%)	
T					
	-	104(100%)	49(100%)	55(100%)	
	+	0	0	0	-
FIIAF					
	-	104(100%)	49(100%)	55(100%)	
	+	0	0	0	-
K					
	-	51(49.04%)	28(57.14%)	23(41.82%)	0.119[b]
	+	53(50.96%)	21(42.86%)	32(58.18%)	
B/O					
	-	102(98.08%)	49(100%)	53(96.36%)	0.497[a]
	+	2(1.92%)	0	2(3.64%)	
F					
	-	34(32.69%)	12(24.49%)	22(40%)	0.092[b]
	+	70(67.31%)	37(75.51%)	33(60%)	

Data are expressed as n(%).
a: Fisher's Exact Test, b:Pearson Chi-Square Test; +: strain carries plasmid replicon; -: strain does not carry any plasmid replicon

Phylogroups

The majority of the strains were grouped as B2 (31.73%) and B1 (22.12%) (Table 4). In strains isolated from cats, group B1 (p= 0.049) and in strains isolated from dogs group E (p=0.025) were statistically significantly more prevalent.

We have shown that the majority of 37 ESBL producing strains were classified in B2 (24.3%-9 strains); carbapenemase-producing strains belonged to C (n=3), B1 (n=3) and E (n=2) phylogroups.However, the relationship between the prevalence of antibiotic resistance rates and phylogroups was found to be statistically not significant (p> 0.05)

There was no statistically significant relationship between the presence of plasmid and genome-encoded integrons and phylogroups (p > 0.05). The majority of strains carrying plasmid-encoded (28.75%)and genome-encoded (31.34%) integron were found to belong to B2 phylogroup.

When we examined the relationship between carrying resistance genes and phylogroups, we focused on carrying the most frequent genes. The majority of CTX-M, TEM and VIM encoding strains were

Table 4. Phylogroups

	Total (n=104)	Cats (n=49)	Dogs (n=55)	p-value
A				
-	97(93.27%)	48(97.96%)	49(89.09%)	0.117[a]
+	7(6.73%)	1(2.04%)	6(10.91%)	
B1				
-	81(77.88%)	34(69.39%)	47(85.45%)	0.049[b]
+	23(22.12%)	15(30.61%)	8(14.55%)	
B2				
-	71(68.27%)	30(61.22%)	41(74.55%)	0.145[b]
+	33(31.73%)	19(38.78%)	14(25.45%)	
C				
-	90(86.54%)	42(85.71%)	48(87.27%)	0.816[b]
+	14(13.46%)	7(14.29%)	7(12.73%)	
E				
-	92(88.46%)	47(95.92%)	45(81.82%)	0.025[b]
+	12(11.54%)	2(4.08%)	10(18.18%)	
F				
-	98(94.23%)	47(95.92%)	51(92.73%)	0.681[a]
+	6(5.77%)	2(4.08%)	4(7.27%)	
UN				
-	95(91.35%)	46(93.88%)	49(89.09%)	0.495[a]
+	9(8.65%)	3(6.12%)	6(10.91%)	

Data are expressed as n(%).
a: Fisher's Exact Test, b:Pearson Chi-Square Test; +: strain belongs phylogroup; -: strain does not belong to phylogroup

grouped in B2 (42%, 34.04% and 28.57%, respectively). There was only a statistically significant relationship between bla_{CTX-M} gene and phylogroup B2 (p=0.030).

DISCUSSION

Human-animal-environment interactions and socio-ecological factors are determinants for the spread of antibiotic resistance in bacteria. It is well known that the irrational usage in human and veterinary medicine and accumulation of antibiotics in the environment lead to emerge and spread of resistant bacteria. Horizontal gene transfer is common among gut bacteria in the gastrointestinal tracts of humans and animals (Goldstein et al., 2001; Costa et al., 2008; Hordijk et al., 2013; Sallem et al., 2013; Jackson et al., 2015; Rocha-Gracia, 2015; Schmidt et al., 2015; Bourne et al., 2019; Ortega-Paredes et al., 2019). Therefore, human and animal microbiota members play a very important role in the spreading of antibiotic resistance (Yousfi et al., 2016; Van Puyvelde et al., 2018; Hernando-Amado et al., 2019; Hong et al., 2019; Lei et al., 2021). Thus, cats and dogs due to their close contact with humans could be important reservoirs for resistant bacteria.

The previous studies were mainly reported antimicrobial resistance of *E. coli* strains obtained from clinical materials of cats and dogs. It has been shown that fecal *E. coli* isolates were mostly resistant to at least one of the antimicrobials tested and their rates were variable. The most common resistance was reported for ampicillin, amoxicillin/clavulanic acid, cephalosporins, tetracycline, trimethoprim and chloramphenicol (Costa et al., 2008; Jackson et al., 2015; Rocha-Gracia et al., 2015; Liu et al., 2016a; Bourne et al., 2019; Chen et al., 2019; Abreu-Salinas et al., 2020; Bandyopadhyay et al., 2021; Lei et al., 2021; Shin et al., 2021)

It has been shown that resistance to AMP (56.73%) followed by CTX (38.46%), FEP (29.81%) and LEV (27.88%) was most prevalent in *E. coli* strains from cats and dogs and there was no statistically significant difference between cats and dogs' strains in our study. The prevalence of ESBL and carbapenemase positivity was 35.5% and 6.7%, respectively. Furthermore, we found that resistance rates for CIP, SXT, LEV, AMP, CTX and FEP were statistically significantly higher in strains isolated from animals that are known to be treated with antibiotics beforehand.

Integrons have important roles in spreading multiple antibiotic resistance between the same and different species by capturing genes in the form of gene cassettes and providing recombination. It was reported that the prevalence of class 1 integrons had a moderate level (11-62%) in cats and dogs' fecal and clinical *E. coli* isolates in several studies (Cocchi et al., 2007; Shaheen et al., 2010; Sallem et al., 2013; Weiss et al., 2018; Ejaz et al., 2021), while class 2 integrons had low percentages (0.8-2%) (Shaheen et al., 2010; Ejaz et al., 2021) or were reported to be not detected (Sallem et al., 2013).

It seems that the prevalences of integrons reported in previous studies were lower than our findings. In our study, class 1 and class 2 integrons were detected as 76.9% and 47.1%, respectively among 104 fecal *E. coli* strains. It was also shown that integrons were encoded mostly on plasmid DNAs (75%) than genomic DNAs (58.65%). Although, previous studies did not report the presence of class 3 integronsin *E. coli* strains isolated from cats and dogs, in our study, we found twelve *E. coli* strains possessed class III integrons. Moreover, dogs strains were more prevalent carriers for integrons.

It is important to show the relationship between integrons and antibiotic resistance especially multidrug resistance phenotypes. Earlier studies from different countries showed that relationships between the presence of integrons and resistance to trimethoprim- sulfamethoxazole, tetracycline (Cocchi et al., 2007; Shaheen et al., 2010; Sallem et al., 2013; Jackson et al., 2015; Rocha-Gracia et al., 2015), sulphonamides (Cocchi et al., 2007; Sallem et al., 2013), beta-lactam antibiotics (Cocchi et al., 2007; Shaheen et al., 2010; Jackson et al., 2015; Ejaz et al., 2021). We did not reach any study investigating the relationship between the presence of integrons and antibiotic resistance in companion animals from Turkey. In accordance with previous studies mentioned above, we found that cat isolates-carrying integrons in plasmid DNAs were found to be resistant to only cefotaxime. Strains carrying- genomicintegrons were found to be resistant to ciprofloxacin, trimethoprim–sulfamethoxazole, ampicillin, ceftazidime, cefepime. Dog isolates carrying integrons on plasmid and genomic DNAs were found to be resistant to trimethoprim-sulfamethoxazole and ampicillin.

The co-existence of integrons and antibiotic resistance genes provides a better understanding of the dissemination of mobile genetic elements among bacterial species. The results indicate that there was a relationship between the presence of beta-lactam resistance genes(Costa et al., 2008; Sallem et al., 2013; Nebbia et al., 2014; Rocha-Gracia et al., 2015; Cui et al., 2017; Alba et al., 2021), trimethoprim-resistance genes (Jackson et al., 2015; Siqueira et al., 2016; Alba et al., 2021) and integrons.

In our study, although there was no statistically significant relationship between antibiotic resistance genes and integrons on plasmid DNAs; genomic class I integrons were found to be statistically significantly related to CTX-M gene.

The prevalences of ESBL determinants were variable (46.6-80%) in clinical and fecal *E. coli* strains isolated from cats and dogs (Carattoli et al., 2005b; Sallem et al., 2013; Nebbia et al., 2014; Schaufler et al., 2015; Yousfi et al., 2016; Gumus et al., 2017; Bortolami et al., 2019; Hong et al., 2019). We found that bla_{CTX-M} (48.08%), bla_{TEM}(45.19%) and bla_{OXA} (4.81%) were the most common genes in all *E. coli* strains. No difference was observed for the prevalence of bla_{CTX-M} and bla_{TEM} genes among dogs and cats; only the bla_{OXA} gene was more prevalent in cats (10.20%, p=0.021). Among 50 CTX-M positive strains, 18 were found to belong to CTX-M group-I and three were found to belong to CTX-M group-IX consistent with previous studies (Gumus et al., 2017; Lei et al., 2021; Shin et al., 2021). ESBL positive strains were shown to carry bla_{CTX-M} (86.4%, p<0.001) and bla_{TEM} genes (67.5%, p<0.001) more prevalently. Consistently with previous studies, we found that bla_{CTX-M} and bla_{TEM} were more frequent than other resistance genes among cats and dogs' fecal *E. coli* strains. Our results proved that healthy companion animals could be a very important carrier for the most common and clinically important resistance genes (bla_{CTX-M} andbla_{TEM}).

There are few studies investigating pAmpC carrying fecal *E. coli* strains obtained from healthy companion animals. Although, the prevalences were low (4-22.7%); *CIT* was the most prevalent pAmpC encoding gene (Hordijk et al., 2013; Rocha-Gracia et al., 2015; Schaufler et al., 2015; Gumus et al., 2017; Shin et al., 2021). Inconsistently with previous findings, we did not find the *CIT* gene; and the frequencies of *DHA* and *MOX* were 3.85% and 2.88%, respectively. It seems that fecal *E. coli* strains do not carry pAmpC β-lactamase as frequently as extended-spectrum β lactamase genes.

It is well known that carbapenemase-producing

bacteria are another concern because treatment options remain very few.There are few studies reporting carbapenemase production in clinical *E. coli* strains from animals; the percentage of bla_{OXA-48} was reported by different authors (Schmiedel et al., 2014; Pulss et al., 2018). Some studies also showed the detection of NDM-5 gene, in clinical *E. coli* isolates (Grönthal et al., 2018; Chen et al., 2019; Hong et al., 2019; Bandyopadhyay et al., 2021). To our knowledge, no studies have been published investigating the carbapenemase producers in the microbiota of healthy cats and dogs in Turkey. According to previous studies, bla_{OXA-48} and bla_{NDM} were the most common genes among carbapenemase-producing *E. coli* strains, which originated from dogs and cats. According to our results, bla_{VIM}(20.1%) was the most common carbapenemase gene and bla_{VIM} gene was found to be statistically significantly (p=0.057) more prevalent in dogs' strains (27.27%) than cats' strains (12.24%). The prevalence of other carbapenemase genes, bla_{OXA-48} (6.73%) and bla_{IMP} (2.88%) were low. It is possible to say that fecal *E. coli* strains do not frequently carry carbapenemase genes yet, consistent with many previous findings (Yousfi et al., 2016; Pulss et al., 2018; Chen et al., 2019). We may think that the carbapenemase resistance pattern is not a concern for companion animals yet but it also should be noted that fecal members have the potential to be a reservoir for carbapenem resistance in the future.

In the last few years, there are some reports from different regions of the world about transmissible colistin resistance via plasmids. According to previous findings, the prevalence of plasmidic colistin resistance genes was lower than other genes, especially, β-lactamase. The low percentages of *mcr* genes (0.08–2.7%) were reported in clinical and fecal *E. coli* isolates from dogs and cats by some authors (Ortega-Paredes et al., 2019; Rumi et al., 2019; Moon et al., 2020; Lei et al., 2021).However, in the present study, we could not detect any positivity for *mcr*genes (*mcr-1 and mcr-2*). It seems that the prevalence of *mcr* genes is low in *E. coli* strains isolated from domestic animals for now and observations should be continued.

Since commensal bacteria in companion animals can carry resistance plasmids, it is important to detect the plasmid replicon types which are related to antibiotic resistance patterns. Previous studies showed that strains isolated from healthy and sick cats and dogs which harbored at least one plasmid replicon type; and it was also reported that InFIB, IncFII, IncF and IncFIA were the most frequent replicon types (Lindsey et al., 2011; Shaheen et al., 2011; Jackson et al., 2015). Moreover, the most common plasmid replicon types were found to encode CTX-M, TEM, CMY-2, NDM-5 and other beta-lactamase and carbapenemase genes in *E. coli* strains obtained from sick and healthy cats and dogs (Tamang et al., 2012; Sallem et al., 2013; Haenni et al., 2014; Siqueira et al., 2016; Kawamura et al., 2017; Ramadan et al., 2020; Alba et al., 2021).

Consistent with previous studies, in our study, 76 strains (73%), were determined to carry more than one plasmid replicon, and some of them had five plasmid replicons. However, 6 *E. coli* strains (5.76%) did not have any of the plasmid replicons investigated. IncF (67.3%), IncK (50.96%), IncFIB (46.15%), IncN (28.85%) and IncI1-Iγ (23.08%) were the most prevalent detected plasmid replicon types in our study. We did not find any statistically significant difference between the plasmid replicon types encoded in isolates of cats and dogs.

Although there was no statistically significant relationship between the presence of CTX-M and TEM genes, these resistance genes were most frequently found in strains carrying IncF, IncF1B, IncK and IncN replicons. On the other hand, there was a statistically significant relationship between bla_{OXA-48} gene and IncN plasmid (p:0.002) and bla_{VIM} gene was found to be related to IncI1-Iγ, IncP and IncB/O plasmid replicons (p: 0.007, p: 0.025, p:0.039, respectively).

Considering the relationship between plasmid replicon types and integrons, a few studies were available. The majority of integron-bearing- *E. coli* strains isolated from cats and dogs were shown to have IncFIA, IncFIB, IncFIC, IncFII, IncI1 IncI2 and IncHI2 plasmid replicon types (Albrechtova et al., 2012; Jackson et al., 2015; Siqueira et al., 2016). Similar to earlier studies, in our study, it was shown that strains carrying integrons had mostly IncF (71.25%, p: 0.018) and IncK (56.25%, p= 0.049) plasmids.

According to previous studies (Schmidt et al., 2015; Liu et al., 2017), healthy cats and dogs' *E. coli* isolates were mostly found to belong to B1 phylogroup; on the other hand, others reported that A phylogroup was the most common (Albrechtova et al., 2012; Carvalho et al., 2016; Yousfi et al., 2016). It seems that the distribution of phylogroups could be variable depending on animal types and/or geographical origin. Bourne et al., 2019 determined that B2 was

the most frequent phylogroup for cats' isolates and B1 group for dogs' strains (Bourne et al., 2019). In the present study, *E. coli* isolates were mainly grouped in B2 (31.73%). Group B1 was statistically significantly more common among cats' strains, group E among dogs' strains.

Previous studies suggested that beta-lactam resistance is related to phylogroups of *E. coli* strains isolated from healthy cats and dogs. According to their findings, the most frequent groups in beta-lactam resistant strains were A and D (Haenni et al., 2014; Schmidt et al., 2015; Yousfi et al., 2016; Aslantaş and Yilmaz, 2017; Liu et al., 2017). However, in our study, ESBL-producing strains mostly belonged to B2 (24.3%), C (21.6%) and B1 (18.9%) phylogroups followed by E (16.2%), F (8.1%) and A (2.7%); carbapenemase-producing strains belonged to C (37.5%), B1 (37.5%) and E (25%) phylogroups.

The correlations of integrons with phylogroups are also important for epidemiological investigations. The majority of integron-carrying *E. coli* strains isolated from cats and/or dogs were shown to belong to A, B1 phylogroups (Skurnik et al., 2006; Sallem et al., 2013; Nebbia et al., 2014; Rocha-Gracia et al., 2015; Siqueira et al., 2016). Other frequent phylogroups were reported to be D and B2 (Skurnik et al., 2006; Cocchi et al., 2007; Sallem et al., 2013; Nebbia et al., 2014; Rocha-Gracia et al., 2015). In our study, strains carrying integrons commonly belonged to B2 phylogroup (in plasmid DNA 28.75%, in genomic DNA 31.34%); nevertheless, it was not found a statistically significant relationship between the presence of integrons and phylogroups.

In several studies, it was shown that the relationships between antibiotic resistance genes and phylogroups were variable. ESBL/pAmpC carrying - fecal *E. coli* strains of cats and dogs were reported to mostly belonging to B1, A and D phylogroups (Sallem et al., 2013; Rocha-Gracia et al., 2015; Schaufler et al., 2015; Ortega-Paredes et al., 2019). On the other hand, carbapenemase positive clinical *E. coli* isolates and *mcr* carrying fecal *E. coli* strains were shown to mostly belonging to B2, D and A phylogroups, respectively (Liu,Thungrat and Boothe 2016; Ortega-Paredes et al., 2019)In our study, strains encoding CTX-M, TEM and VIM genes frequently belonged to B2, B1, E and C phylogroups; the relationship between the presence of bla_{CTX-M} gene and B2 phylogroup was shown to be statistically significant (p:0.030).

CONCLUSION

Our results showed that fecal *E. coli* strains with different plasmid replicon types and from various phylogroups isolated from cats and dogs were resistant to various antibiotics which are used in human and veterinary medicine. Furthermore, *E. coli* strains investigated harbor plasmid-mediated resistance genes and integrons which are responsible for the transmission of antibiotic resistance between different bacteria in different habitats. Considering that human-animal-environment interactions are major determinants for the spread of antibiotic resistance, our results proved that microbiota members of healthy animals could be a very important reservoir for the most common resistance genes. Therefore, it is obvious that the antibiotic resistance problem should be approached in correlation with the "One Health" concept to avoid the spread of antibiotic resistance between wildlife-, livestock - and domestic animals, humans and the environment.

CONFLICT OF INTEREST

There is no conflict of interest to disclose

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