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Investigation of antimicrobial resistance in pigeons (*Columba livia domestica*) using indicator bacteria

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ABSTRACT: The aim of this study was to determine the prevalence of antibiotic resistance as well as presence of resistance-associated genes in *Escherichia coli* and *Enterococcus* spp. strains isolated from pigeons. One hundred and fifty cloacal swabs were collected from apparently healthy pigeons in Hatay, Turkey, between March 2014 and June 2014. Antimicrobial susceptibilities of the isolates were tested with disc diffusion method, and resistance genes were investigated by polymerase chain reaction (PCR). *E. coli* were isolated from 94.7% (142) of the examined cloacal swab samples. *E. coli* isolates revealed higher resistance rates to tetracycline (51.4%) and ampicillin (50%), followed by nalidixic acid (19.7%), streptomycin (12.7%), amoxycillin-clavulanic acid (15.5%), trimethoprim-sulfamethoxazole (10.6%), cephalothin (7.0%), ciprofloxacin (6.3%), kanamycin (4.9%), gentamicin (4.2%), tobramycin (4.2%), ceftazidime (4.2%), cefotaxime (4.2%), chloramphenicol (2.8%), aztreonam (2.8%), and cefoxitin (0.7%), respectively. Twentyeight (%19.7) *E. coli* isolates were susceptible to all tested antimicrobials. A total of 136 (90.7%) *Enterococcus* spp. were isolated and species distribution of the isolates was determined by species-specific PCR. The isolates were identified as 64 (47.1%) *E. hirae*, 17 (12.5%) *E. faecium*, 8 (5.9%) *E. faecalis*, 4 (2.9%) *E. columbea*, and 2 (1.5%) *E. durans*. The rest of the isolates (30.1%) were identified as *Enterococcus* spp. with the used primers. *Enterococcus* spp. were resistant to tetracycline (67.6%), erythromycin (23.5%), rifampicin (17.6%), chloramphenicol (6.6%) and ciprofloxacin (5.9%). By contrast, 38 (27.9%) *Enterococcus* spp. were sensitive to all tested antimicrobials. The data obtained in the study showed that pigeons were carriers of antimicrobial resistant *E. coli* and *Enterococcus* spp. in their intestinal microbiota, and may pose public health risk due to not only transmission of these resistant bacteria to humans but also contamination of the environment. The current status of antimicrobial resistance in different animal species should be continuosly monitored and control measures should also be taken.

Keywords: Pigeon, antimicrobial resistance, *Escherichia coli*, *Enterococcus* spp.

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INTRODUCTION

Bacterial resistance to antimicrobials are growing problem in both human and veterinary medicine worldwide. The main risk factor for the emergence of resistant bacteria is misuse and overuse of antibiotics (van den Bogaard and Stobberingh, 2000). Pigeons can not only play an important role for the dissemination of zoonotic agents such as chlamydiosis, cryptococcosis, aspergillosis and can also host antimicrobial resistant bacteria such as *Escherichia coli*, *Campylobacter* spp., *Salmonella* spp. and *Enterococcus* spp. (Vasconcelos et al., 2018; Perez-Sancho et al. 2020). Oral administration of various antibiotics for prophylactic and therapeutic purposes causes selective pressure on the microbiota and leads the selection of resistant bacteria (Mehdi et al. 2018). Tetracyclines and beta-lactam antibiotics are widely used for the treatment of poultry infections due to its low cost, efficacy, and lack of side effects (Filazi et al. 2017)

E.coli and *Enterococcus* spp. are commensal inhabitants of gastrointestinal flora of animals, and have been used as a indicator bacteria not only for faecal contamination of environment and but also of food, in particular, monitoring antimicrobial resistance in different animal species (Kojima et al., 2009; Persoons et al., 2010; Radimersky et al., 2010). In additon to being a potential reservoir for resistance genes, indicator bacteria are of particular importance because they can transfer resistance genes to other bacterial populations either with in the same or other any host. Indicator bacteria have also important role for giving an overview of the resistance load of the ecosystem in which they are in (Wray and Gnanou, 2000). Antimicrobial resistance in bacteria occured by intrinsic or acquired mechanisms. Acquired resistance occurs due to different mechanisms in bacteria: (i) target mutation, (ii) acquisition of resistance genes located on mobile transmissible elements such as plasmids, transposoons, and integrons via conjugation, transduction and transformation (Munita and Arias, 2016).

Recent studies have shown that both free-living pigeons and domesticated pigeons are potential reservoirs of resistant bacteria (Radimersky et al., 2010; Aşkar et al., 2011; Blanco-Peña et al., 2017). Due to the fact that pigeons are close proximity to humans and its impact on public health, it is important to investigate the antimicrobial resistance in pigeons using indicator bacteria. In Turkey, pigeon keeping and breeding on the roof of the houses are a common hobby. However, the data on carriage of antimicrobial resistance in their gastrointestinal flora is very limited (Aşkar et al., 2011). Therefore, the objectives of this study were to investigate the occurence of antimicrobial resistance in indicator bacteria in faeces of pigeons and the mechnanisms mediating resistance.

MATERIAL AND METHODS

Ethical statement

The study was approved by the Animal Ethical Committee of Hatay Mustafa Kemal University (2013-7/7).

Sampling

A total of 150 cloacal swab samples were collected from the houses belonging to people dealing with pigeon breeding as a hobby in three locations in Hatay, Turkey, between March 2014 and June 2014. For this purpose, five pigeon premises from each settlement were sampled, and the cloacal swab samples were taken from 10 pigeons from each premises.

Isolation of *E. coli* strains

Individual cloacal swab samples were taken by Stuart Transport Medium and transported to laboratory in cold chain. For *E. coli* isolation, cloacal swab samples were directly inoculated onto Eosin Methylene Blue (EMB) agar and incubated at 37 ^{o}C for 24 h. Following biochemical tests, the isolates were confirmed by polymerase chain reactions (PCR) using *E. coli* species specific primers E16S-F 5'-CCC CCT GGA CGA AGA CTG AC-3 'and E16S-R 5'-ACC GCT GGC AAC AAA GGA TA-3' (Wang et al., 2002).

Antimicrobial susceptibility testing and detection of resistance genes of *E. coli* isolates

Antimicrobial susceptibilities of *E. coli* isolates to nineteen antimicrobials were determined by disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI, 2012) guidelines. The antimicrobial disks (Bioanalyse, Turkey) used were: ampicillin (AMP, 10 μg), amoxycillin-clavulanic acid (AMC,20/10 μg), nalidixic acid (NA, 30 μg), ciprofloxacin (CIP, 5 μg), cefpodoxim (CPD, 10 μg), ceftriaxone (CRO, 30 μg), cefepime (FEB, 30 μg), cefoxitin (FOX, 30 μg), cefuroxime (CXM, 30 μg), cephalothin (KF, 30 μg), aztreonam (ATM, 30 μg), imipenem (IMP, 10 μg), chloramphenicol (C, 30 μg), gentamicin (CN, 10 μg), tobramycin (TOB, 10 μg), amikacin (AK, 10 μg), kanamycin (K, 30 μg), tetracycline (TE, 30 μg), and

sulphamethoxazole-trimethoprim (SXT, 1.25/23.75 µg). *E. coli* ATCC 25922 strain was used as control strain for antimicrobial susceptibility testing. The isolates showing resistance to three or more antimicrobials from different classes were defined as multidrug resistant (MDR). Penicillins and cephalosporins were considered as separate classes. The isolates showing resistance to 3rd generation cephalosporins were confirmed as extended spectrum beta-lactamase (ESBL) producer by double disk synergy (Jarlier et al., 1988) and disk combination method according to guidelines of CLSI (2012).

The isolates showing resistance to particular antibiotics were screened for the presence of antibiotic resistance genes in *E. coli* by PCR using the primers listed in Table 1.

Table 1. Primers used for detection of antibiotic resistance genes in *E. coli* isolates

Antibiotics	Gene	Sequence (5'-3')	Product Size (bp)	Reference
Tetracyclines	*tet*(A)	GCTACATCCTGCTTGCCTTC CATAGATCGCCGTGAAGAGG	210	Ng et al. (2001)
	tet(B)	TTGGTTAGGGGCAAGTTTTG GTAATGGGCCAATAACACCG	659	
	tet(C)	CTTGAGAGCCTTCAACCCAG ATGGTCGTCATCTACCTGCC	418	
	tet(D)	AAACCATTACGGCATTCTGC GACCGGATACACCATCCATC	787	
	tet(E)	AAACCACATCCTCCATACGC AAATAGGCCACAACCGTCAG	278	
	tet(G)	GCTCGGTGGTATCTCTGCTC AGCAACAGAATCGGGAACAC	468	
Chloramphenicol	*catI*	AGTTGCTCAATGTACCTATAACC TTGTAATTCATTAAGCATTCTGCC	547	Maynard et al. (2004)
	catII	ACACTTTGCCCTTTATCGTC TGAAAGCCATCACATACTGC	543	
	catIII	TTCGCCGTGAGCATTTTG TCGGATGAGTATGGGCAAC	286	
Trimethoprim	*dhfrI*	AAGAATGGAGTTATCGGGAATG GGGTAAAAACTGGCCTAAAATTG	391	Maynard et al. (2004)
	dhfrV	CTGCAAAAGCGAAAAACGG AGCAATAGTTAATGTTTGAGCTAAAG	432	
	dhfrVII	GGTAATGGCCCTGATATCCC TGTAGATTTGACCGCCACC	265	
	dhfrIX	TCTAAACATGATTGTCGCTGT C TTGTTTTCAGTAATGGTCGGG	462	
	dhfrXIII	CAGGTGAGCAGAAGATTTTT CCTCAAAGGTTTGATGTACC	294	
Aminoglycosides	*aadA*	GTGGATGGCGGCCTGAAGCC AATGCCCAGTCGGCAGCG	525	Kozak et al. (2009)
	strA/*strB*	ATGGTGGACCCTAAAACTCT CGTCTAGGATCGAGACAAAG	893	
	aac(3)IV	TGCTGGTCCACAGCTCCTTC CGG ATGCAGGAAGATCAA	653	
	aadB	GAGGAGTTGGACTATGGATT CTTCATCGGCATAGTAAAAG	208	
	aphA1	ATGGGCTCGCGATAATGTC CTCACCGAGGCAGTTCCAT	600	
	aphA2	GATTGAACAAGATGGATTGC CCATGATGGATACTTTCTCG	347	

Sulphanamid	*sul1*	CGGCGTGGGCTACCTGAACG GCCGATCGCGTGAAGTTCCG	433	Kozak et al. (2009)
	sul2	CGGCATCGTCAACATAACCT TGTGCGGATGAAGTCAGCTC	721	
	sul3	CAACGGAAGTGGGCGTTGTGGA GCTGCACCAATTCGCTGAACG	244	
β-lactams	bla_{SHV}	ATGCGTTATATTCGCCTGTG TGCTTTGTTATTCGGGCCAA	747	Monstein et al. (2007)
	bla_{TEM}	TCGCCGCATACACTATTCTCAGAATGA ACGCTCACCGGCTCCAGATTTAT	445	
	bla_{CTX}	ATGTGCAGYACCAGTAARGTKATGGC TGGGTRAARTARGTSACCAGAAAYCAGCGG	593	
	bla_{CMY-2}	GACAGCCTCTTTCTCCACA TGGAACGAAGGCTACGTA	1015	Zhao et al. (2001)
Quinolones	*qnr*A	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	516	Kim et al., 2009
	*qnr*B	GATCGTGAAAGCCAGAAAGG ATGAGCAACGATGCCTGGTA	416	
	*qnr*C	GGGTTGTACATTTATTGAATCG CACCTACCCATTTATTTTCA	307	
	*qnr*S	GCAAGTTCATTGAACAGGGT TCTAAACCGTCGAGTTCGGCG	428	
	aac(6')-Ib-cr	TTGCGATGCTCTATGAGTGGCTA CTCGAATGCCTGGCGTGTTT	482	Park et al., 2006

***Enterococcus* spp. isolation and species determination using PCR**

Cloacal swab were firstly inoculated into Enterococcosel Broth (BD, USA) and incubated at 37 °C for 24 h. In case of colour change, a loopful of culture was plated onto VRE agar. Plates were incubated at 37 °C for 24 h, and then one typical colony was selected and passaged to blood agar plates supplemented with 5% defibrinated sheep blood in order to obtain pure culture. The isolates were identified on the genus level by Gram staining, catalase tests. Determination of *Enterococcus* spp. on genus and species level were done by using primers and method described by Layton et al. (2010), except *E. columbae*, which was examined as previously described by da Silva et al. (2012).

Antimicrobial susceptibility testing and detection of resistance genes of *Enterococcus* spp.

Antimicrobial susceptibilities of the isolates to eight antimicrobials were determined by disk diffusion method in accordance with CLSI (2012) criteria, and the used disks were as follow: ampicillin (AMP, 10 μg), vancomycin (VA, 30 μg), erythromycin (E, 15 μg), tetracycline (TE, 30 μg), teicoplanin (TEC, 30 μg), ciprofloxacin (CIP, 5 μg), and chloramphenicol (C, 30 μg). For the phenotypic determination of high level gentamicin resistance (HLGR), 120 μg gentamicin containing disks were used. The isolates showing resistance to particular antibiotics were screened for the presence of antibiotic resistance genes in enterococci by PCR using the primers listed in Table 2.

Table 2. Primers used for detection of antibiotic resistance genes in enterococci

Antibiotic	Primer	Sequence (5'-3')	Product size (bp)	Reference
Macrolides	*erm*(A)	CCCGAAAAATACGCAAAATTTCAT CCCTGTTTACCCATTTATAAACG	590	Malhotra-Kumar et al. (2005)
	erm(B)	TGGTATTCCAAATGCGTAATG CTGTGGTATGGCGGGTAAGT	745	
	mef(A/E)	CAATATGGGCAGGGCAAG AAGCTGTTCCAATGCTACGG	317	
Tetracycline	*tet*(K)	GATCAATTGTAGCTTTAGGTGAAGG TTTTGTTGATTTACCAGGTACCATT	155	
	tet(M)	GTGGACAAAGGTACAACGAG CGGTAAAGTTCGTCACACAC	406	
	tet(O)	AACTTAGGCATTCTGGCTCAC TCCCACTGTTCCATATCGTCA	515	
	tet(L)	TGGTGGAATGATAGCCCATT CAGGAATGACAGCACGCTAA	229	
Aminoglycosides	*aac(6)-Ie-aph(2)-Ia*	CAGGAATTTATCGAAAATGGTAGAAAAG CACAATCGACTAAAGAGTACCAATC	369	Vakulenko et al. (2003)
	aac(6)-Ie-aph(2)-Ia	CAGAGCCTTGGGAAG ATG AAG CCTCGTGTAATTCATGTTCTGGC	348	
	aph(2)-Ib	CTTGGACGCTGAGATATATGAGCA C GTTTGTAGCAATTCAGAAACACCCTT	867	
	aph(2)-Ic	CCA CAATGATAATGACTCAGTTCCC CCA CAGCTTCCGATAGCAAGAG	444	
	aph(2)-Id	GTG GTTTTTACAGGAATGCCATC CCCTCTTCATACCAATCCATATAACC	641	
	aph(3)-IIIa	GGCTAAAATGAGAATATCACCGG CTTTAAAAAATCATACAGCTCGCG	523	
	ant(4)-Ia	CAAACTGCTAAATCGGTAGAAGCC GGAAAGTTGACCAGACATTACGAACT	294	
Chloramphenicol	*Cat*pIP 501-159-	GGATATGAAATTTATCCCTC CAATCATCTACCCTATGAAT	505	Aerestrup et al. (2000)
Vancomycin	*vanA*	GGGAAAACGACAATTGC GTACAATGCGGCCGTTA	732	Depardieu et al. (2002)
	vanB	ACGGAATGGGAAGCCGA TGCACCCGATTTCGTTC	647	
	vanC1/2	ATGGATTGGTAYTKGTAT TAGCGGGAGTGMCYMGTAA	815/827	
	vanD	TGTGGGATGCGATATTCAA TGCAGCCAAGTATCCGGTAA	500	
	vanE	TGTGGTATCGGAGCTGCAG ATAGTTTAGCTGGTAAC	430	
	vanG	CGGCATCCGCTGTTTTTGA GAACGATAGACCAATGCCTT	941	

RESULTS

E. coli isolation and antimicrobial testing

One hundred and forty two (94.7%) *E. coli* were isolated from 150 cloacal swab samples. Various rates of resistance among *E. coli* isolates were observed to tetracycline (73, 51.4%), ampicillin (71, 50%), nalidixic acid (28, 19.7%), amoxycillin-clavulanic acid (22, 15.5%), streptomycin (18, 12.7%), trimethoprim-sulfamethoxazole (15, 10.6%), cephalothin (10, 7.0%), ciprofloxacin (9, 6.3%), kanamycin (7, 4.9%), gentamicin (6, 4.2%), tobramycin (6, 4.2%), ceftazidime (6, 4.2%), cefotaxime (6, 4.2%), chloramphenicol (4, 2.8%), aztreonam (4, 2.8%), and cefoxitin (1, 0.7%), respectively (Figure 1). Twentyeight (19.7%) isolates were found susceptible to all antimicrobials tested. Twentyseven (19%) isolates showed MDR phenotype. Among the isolates showing MDR phenotype, resistance to 6, 5, 4, and 3 isolates were observed in 2, 3, 8, and 14 isolates, respectively (Table 3).

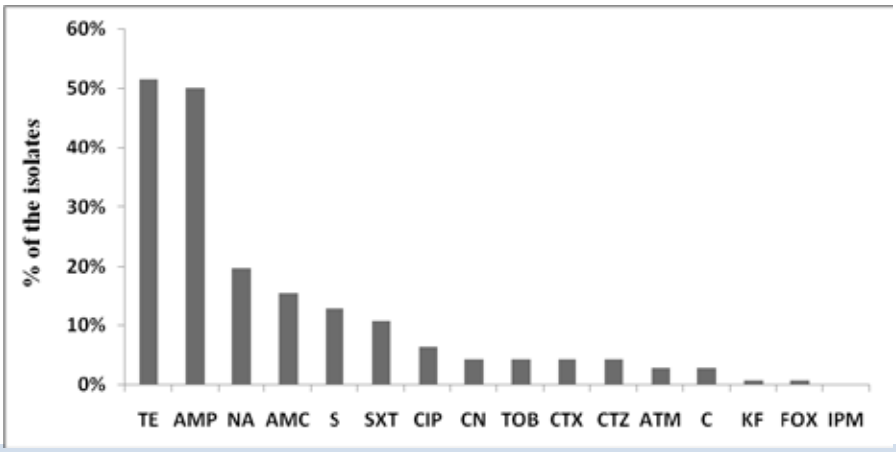

Figure 1. Antibiotic susceptibilities of 142 *E. coli* isolates

Table 3. Antibiotic resistance phenotypes among the *E. coli* isolates

Phenotype	Number of the isolates
AM, AMC, KF, TE, CN, S, K, TOB, SXT	1
AM, KF, TE, K, TOB, SXT, CIP NA, C	1
AM, TE, K, TOB, SXT, CIP, NA, C	1
AM, TE, CN, S, K, TOB, SXT	1
AM, TE, CN, TOB, CIP, NA, C	1
AM, AMC, TE, CN, S, K, SXT	1
AM, AMC, TE, S, K, SXT	1
AM, AMC, KF, TE, NA	2
AM, TE, SXT, CIP, NA	2
CN, TOB, CIP, NA, C	1
AM, TE, S, SXT, NA	2
AM, TE, CN, K, SXT	1
AM, AMC, TE, S, K	1
AM, AMC, KF, TE	1
AM, SXT, CIP, NA	2
AM, KF, TE, NA	1
AM, TE, S, SXT	1
AM, AMC, TE	6
AM, AMC, KF	3
AM, TE, S	4
AM, KF, TE	1
TE, S, NA	3
AM, AMC	1
AM, CIP	1
TE, SXT	1
CIP, NA	1
AM, TE	20
TE, NA	8
AM, S	1
TE, S	1
AM	15
TE	18
NA	7
KF	1
S	1
Susceptible	28

Table 4. Antibiotic resistance and resistance mechanisms of *Enterococcus* spp.

Phenotype	Resistance Genes	Species (n)
C, CIP, E, RA, TE	*cat*, *tet*M, *tet*L, *erm*B	*E. faecium* (1)
CIP, E, RA, TE	*tet*M, *tet*L, *erm*B	*Enterococcus* spp. (1)
C, E, RA, TE	*cat*, *tet*M, *tet*L, *erm*B	*E. faecium* (1)
CIP, E, RA	*erm*B	*E. faecium* (1)
CIP, E, TE	*tet*M, *tet*L, *mef*A/E	*E. columbea* (1), *E. faecium* (2)
E, RA, TE	*tet*M, *erm*B	*Enterococcus* spp. (2)
E, RA, TE	*tet*M, *tet*L, *mef*A/E	*Enterococcus* spp. (1)
E, RA, TE	*tet*M, *tet*L, *erm*B	*E. hirae* (2)
C, E, TE	*tet*M, *tet*L, *erm*B	*Enterococcus* spp. (2), *E. hirae* (1)
C, E, TE	*tet*M, *tet*L	*E. hirae* (1)
RA, TE	*tet*M, *tet*L	*Enterococcus* spp. (5), *E. faecalis* (1), *E. faecium* (1), *E. hirae* (4)
RA, TE	*tet*M	*E. faecium* (1), *E. hirae* (1)
CIP, E	-	*E. columbea* (1)
C, TE	*tet*M, *tet*L	*Enterococcus* spp. (1)
C, TE	*tet*M	*E. hirae* (1)
E, TE	*tet*M, *tet*L, *erm*B	*Enterococcus* spp. (3)
E, TE	*tet*M, *erm*B	Enterococcus spp. (1)
E, TE	*tet*L	*Enterococcus* spp. (1), *E. faecium* (1), *E. hirae* (1)
E, TE	*tet*M, *tet*L, *erm*B	*Enterococcus* spp. (1), *E. faecium* (1), *E. hirae* (2)
E, TE	*tet*M, *tet*L, *mef*A/E	*Enterococcus* spp. (1), *E. faecium* (1), *E. hirae* (2)
TE	*tet*M, *tet*L	*E. hirae* (2)
TE	*tet*M, *tet*L	*Enterococcus* spp. (4), *E. columbea* (2), *E. faecium* (1), *E. hirae* (3)
TE	*tet*M	*Enterococcus* spp. (6), *E. hirae* (26)
TE	*tet*L	*E. hirae* (1)
TE	-	*E. hirae* (2)
RA	-	*E. faecalis* (2)
CIP	-	*E. faecium* (1)
C	-	*E. hirae* (1)
Sensitive	-	*Enterococcus* spp. (12), *E. durans* (2), *E. faecalis* (5), *E. faecium* (5), *E. hirae* (14)

Distribution of resistant genes among resistant *E. coli* isolates

Tetracycline resistance was only associated with *tet*A and *tet*B genes, which were found in 77 (95.1%) of 81 tetracycline resistant *E. coli* isolates. The distribution of resistance genes were as follows: 62 (80.5%) *tet*A, 14 (18.2%) *tet*A and *tet*B, and one (1.3%) *tet*B. All isolates were negative for *tet*C, *tet*D, *tet*E and *tet*G.

Among ampicillin resistant isolates, bla_{TEM} was found in 66 (91.7%) isolates. PMQR genes were detected in four ciprofloxacin resistant isolates, of which three isolates carried *aac(6')-Ib-cr*, and one carried *qnr*A. Among trimethoprim-sulfamethoxazole resistant isolates (n=15), the distribution was determined as follows: *sul*1-*sul*2 in four isolates, *sul*1-*sul*2-*dhfr*1 in two isolates, *sul*1-*dhfr*1 in two isolates, *sul*2-*dhfr*5 in two isolates, *sul*1 in two isolates, *sul*1 in two isolates, and *sul*1-*sul*2-*dhfr*5 in one isolate. While all ESBL producing *E. coli* isolates carried bla_{CTX-M}, bla_{C-MY-2} gene was only detected in one cefoxitin isolate.

Of 18 streptomycin resistant isolates, 15 (83.3%) carried *str*A/B. Three isolates didn't carry any of the genes examined. Out of four chloramphenicol resistant isolates, only 3 (75%) carried *cat*I. Of kanamycin resistant eight isolates, *aph*A1 was only detected in 6 (75%) isolates. The *aad* and *aac*(3)IV genes were not detected in any tobramycin and gentamicin resistant isolates.

Isolation, species determination and antimicrobial susceptibility of *Enterococcus* spp.

Enterococcus spp. were isolated 136 (90.7%) from pigeon's cloacal swabs. Based on species spesific PCR, distribution of enterococci were as follow: 64 (47.1%) *E. hirae*, 17 (12.5%) *E. faecium*, 8 (5.9%) *E. faecalis*, 4 (2.9%) *E. columbea*, and 2 (1.5%) *E.*

durans. However, 41 (30.1%) isolates were only detected as *Enterooccus* spp. with current primers used.

Antibiotic resistance rates of 136 enterococci were 67.6% (92) to tetracycline, 23.5% (32) to erythromycin, 17.6% (24) to rifampicin, 6.6% (9) to chloramphenicol, and 5.9% (8) to ciprofloxacin. Thirty-eight (27.9%) isolates were sensitive to all tested antimicrobials. Resistance phenotypes and resistance-mediated genes in enterococcal isolates are shown in Table 4. MDR phenotype was observed in 16 (11.8%) isolates. Among the isolates showing MDR phenotype, resistance to 5, 4, and 3 antimicrobials was observed in one, two and thirteen isolates, respectively.

DISCUSSION

Pigeons not only freely lives in urban and rural areas, but also they were raised by people as a hobby. In addition, pigeons are in close contact with humans in different public locations, such as historical places, parks, and squares. These birds may pose possible risks to public health due to carriage of different zoonotic microorganisms (bacteria, fungi, viruses, and protozoa) and antimicrobial resistant bacteria (Vasconcelos et al., 2018; Perez-Sancho et al. 2020).

In this study, 80.3% of the *E. coli* isolates were resistant to one or more antimicrobials tested. In other conducted studies on the occurence of antimicrobial resistant *E. coli* isolates in pigeons, low or lower rates of resistance in *E. coli* isolates have been reported by Radimersky et al. (2010) in Czech Rebuplic (1.5%) and da Silva et al. (2009) in Brazil (37.9%), respectively.

Nineteen percent (n=27) of *E. coli* isolates showed MDR phenotype. MDR bacteria are an increasing an healthcare problem because the presence of pathogens with MDR phenotype, making treatment options very limited. The fact that co-existence of resistance genes on transmissible genetic elements such as plasmid and transposon, facilitate horizantal transfer of resistance genes to susceptible bacteria and lead to an expansion in MDR bacteria population. Therefore, continuous surveillance of antimicrobial resistance in different animal species and environments are important for taking timely necessary measures (Frye and Jackson, 2013)

Resistance to tetracycline (51.4%) and ampicillin (50%) were the most prevalent among the isolates in this study, which are consistent with the findings of Kimpe et al. (2002), who reported resistance rates of 65% and 42%, respectively. However, in Poland, Stenzel et al. (2014) reported a higher resistance rate for amoxicillin (63%) and oxytetracycline (75%), respectively. The *tet*A was the most common resistance gene in comparison with other resistance genes in the study. High prevalence of *tet*A among the tetracycline resistant isolates also indicates that the main resistance mechanism is the active efflux system (Blake et al., 2003). There are few studies on prevalence of antimicrobial resistance genes in pigeons around the world. Blanco-Pena et al. (2017) found *sul*1 and *cat*1 as the most common gene by real time PCR from directly enema samples of pigeons from Public Parks in Costa Rica. In Iran, Ghanbarpour et al. (2020) reported phenotypically the prevalence of tetracycline resistance as very high (98%), but detected a lower prevalence of *tet*A (6.5%) and *tet*B (6.5%) genes.

Nearly all ampicillin resistant isolates carried bla_{TEM} gene (91.7%, 66/72), which was the second most common gene found in the study. In contrast, in Iran, bla_{TEM} was reported to be the most common gene (52.6%) by Ghanbarpour et al. (2020). Similarly, the TEM type beta-lactamase has also been reported as main resistance mechanism of ampicillin resistance in *E. coli* isolates from different origin of animals in previously conducted studies (Radhouani et al., 2012; Santos et al., 2013; Aslantaş, 2018).

Sulfanamids and trimethoprim are folate patway inhibitors, and main resistance mechanisms to these antimicrobials are due to mutations in target enzymes, encoded by *sul* and *dhfr* genes (Skold, 2001). Trimethoprim-sulfamethoxazole resistant isolates had a combination of *sul* and *dhfr* genes, except four isolates which carried only *sul*1 and *sul*2 genes. None of the isolates harbored *sul*3, *dhfr7, dhfr9* and *dhfr13*. Recently, Aslantaş (2018) reported not only high sulfanamid and trimethoprim resistance but also high frequency of these resistant genes among commensal *E. coli* isolates from broilers in Turkey. Widespread dissemination of the resistance genes in *E. coli* could be explained by localization of these genes on plasmids, integrons, or insertion elements (Frye and Jackson, 2013).

Aminoglycoside resistance in *E. coli* strains are mainly related with aminoglycoside modifying enzymes, which is encoded by genes located on plasmids (Frye and Jackson, 2013). Low rate of aminoglycoside resistance is not surprising, because these drugs are not widely used in veterinary field in Turkey. Similarly, Ghanbarpour et al. (2020) reported a

low prevalence of resistance (11%) for gentamicin. Occurence of low resistance might be originated from contaminated feeds and their environments of the pigeons (Radimersky et al., 2010).

Low level of ciprofloxacin resistance was observed in this study. This is important due to the fact that fluoroquinolones are critically important antimicrobials used for the treatment of *E. coli* infections (WHO, 2012). The ciprofloxacin resistance rate is consistent with previous studies conducted by Radimersky et al. (2010) and Aşkar et al. (2002), who reported resistance rates of 2% and 0%, respectively.

Resistance to 3rd and 4th generation cephalosporins mediated by ESBL have clinical importance for both human and veterinary medicine (WHO, 2012). Prevalence of ESBL producing *E. coli* isolates was found to be low in this study. It should be cautiously approached to low rate of resistance. Because selective isolation methods are needed to determine the true prevalence of these bacteria in different animal species (Aslantaş, 2018).

Although 41 (30.1%) isolates were assigned as *Enterococcus* spp. with current primers used in this study. The most common species were identified as *E. hirae* (47.1%), followed by *E. faecium* (12.5%), and *E. faecalis* (5.9%), respectively. *E. columbea* (2.9%) and *E. durans* (1.5%) were detected only in small number of the isolates. In Belgium and Brazil, *E. columbea* was reported as the most frequent species by Baele et al. (2002) and da Silva et al. (2012), respectively. Radimersky et al. (2010) reported that *E. faecalis* and *E. faecium* were as the most frequent species among enterococci isolated from feral pigeons in Czech Republic. Aşkar et al. (2011) reported *E. avium* as most prevalent species among enterococci from domestic pigeons. In a recent study, *E. faecium* and *E. durans* were reported as dominant species in pigeons in Egypt by Osman et al. (2019). Species distribution of enterococci in pigeon in different geographies could be explained by dietary habits of pigeons, which leads colonization of pigeon with different enterococci (Beale et al., 2002).

Although enterococci can exhibit intrinsic resistance to different classes of antimicrobials at low or high levels, they can frequently acquire antimicrobial resistance to different class of antimicrobials such as high-level aminoglycoside resistance (HLAR), fluoroquinolones, glycopeptides, and beta-lactams (ampicillin), via mutations or acquisition of resistance genes (Marothi et al., 2005). The prevalence of antimicrobial resistance in enterococci (72.1%, 98/136) was higher in comparison with previous studies in pigeons, and tetracycline resistance were the most prevalent type of resistance, and were mainly associated with *tet*M. Similar resistant rate (78%) and resistance determinant were also reported by Radimersky et al. (2010) in Czech Republic. Recently, Zigo et al. (2017) found both higher prevalence of antimicrobial resistant enterococci and high resistance rate to tetracycline (75.2%) in Slovakia. In this study, the high observed tetracycline resistance can be attributed to empirical use of this antibiotic for many years by pigeon owners.

The second most common resistance observed was to erythromycin (23.5%), mainly associated with *erm*B gene (79.2%). In contrast, Aşkar et al. (2011) and Zigo et al. (2017) reported higher resistance rate for erythromycin (52%) and 52.2%, respectively. However, a low resistance rate was reported by Radimersky et al. (2010) in Czech Republic, who found a resistance rate of 9% for erythromycin. Interestingly, Osman et al. (2019) found resistance rates ranging from 63.4% and 100% for antibiotics tested, except linezolid (17.1%), in enterococci in Egypt.

Low rate resistance to chloramphenicol (6.6%) among enterococci in this study is not surprising. Since the use of chloramphenicol was banned in food producing animals in Turkey (Regulation No: 2002/68 of 19 December 2002). Low rate resistance to this drug could be explained by the persistence of chloramphenicol resistant strains in the environment (Persoons et al., 2010) or co-existence of chloramphenicol resistance genes with other resistance genes on the same mobile genetic elements (Harada et al., 2006). However, in contrast with this study, da Silva et al. (2009) reported a higher resistance rate (21.7%) in Brazil.

Main resistance mechanism to fluoroquinolones in enterococci is characterized by mutations in the quinolone determining regions of *gyr*A and *par*C genes. The level of resistance to fluoroquinolones varies according to the intensity and duration of use of these antimicrobials. Indeed, in countries where the use of fluoroquinolones is prohibited in food-producing animals, no or low resistance rates can be accepted as an indication of this view (Cheng et al., 2012). Ciprofloxacin resistance rate (5.9%) observed in this study was consisted with previous studies conducted by da Silva et al. (2012) in Brazil and Radimersky et

al. (2010) in Czech Rebuplic, who reported resistance rates of 8.4% and 5%, respectively. But, Aşkar et al. (2011) found higher resistance rate (37%) in Kırıkkale, Turkey. The low resistance rate observed in this study was due to low level empirical use of this drug by pigeon owners for the treatment or prevention of infectious diseases.

One of the striking results of the study was no resistance against high level gentamicin and vancomycin. Gentamicin is one of the antimicrobials having clinical importance. Because combination of this drug with beta-lactams have been widely used for the treatment of enterococcal infections. However, this combination is ineffective in the treatment of infections caused by enterococci with HLGR resistance (del Campo et al., 2000). Vancomycin is a last resort antibiotic to be used for the treatment of nosocomial infections caused by Gram positive bacteria. Similarly, no vancomycin resistance was reported by Silva et al. (2012) in Brazil, Blanco-Peña et al. (2017) in Costa Rica and Aşkar et al. (2011) in Turkey. However, Radimersky et al. (2010) in Czech Rebuplic reported vancomycin resistance in three *E. faecalis* isolates (2%) carrying *van*A gene. In a study conducted in Egypt, Osman et al. (2020) reported higher level (40/41, 97.6%) of VRE colonization and detected frequency of *van*A, *van*B and *van*C genes as 17.1%, 24.4%, and 22%, respectively

CONCLUSIONS

In conclusion, various rates of resistance to different classes of antimicrobials in *E. coli* and *Enterococcus* spp. isolates from the faeces of pigeons were observed in this study. These findings are important not only due to spreading of resistant bacteria to environment and susceptible animals, but also transfer of resistance genes to pathogenic bacteria. Based on the results of this study, there is an urgent need to investigate the antimicrobial resistance in different animal species, and to promote prudent use of antimicrobials for the treatment and control of bacterial infections.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest

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