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Original Article

Antimicrobial Resistance of *E. coli* and *Salmonella* Isolated from Wild Birds in a Rehabilitation Center in Turkey

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Abstract

Wildlife plays a critical role as a reservoir for zoonosis especially pathogenic enteric bacteria. In this study we evaluated the presence of *E. coli* and *Salmonella* isolates from wild birds and determined their antimicrobial resistance. Intestine and fecal samples from 82 dead wild birds obtained from rehabilitation centre**,** were examined by microbiological analysis, antibiotic susceptibilities against of 18 antimicrobials and presence of tetracycline resistance genes by multiplex and singleplex PCR were investigated. A total of 51 *E. coli* were identified as well as *Salmonella* Kentucky and *Salmonella* Bisberg. A majority of the *E. coli* isolates were resistant to lincomysin (100%), penicilline (96.1%), kanamycin (80.4%), tetracycline (68.6%), and oxytetracycline (64.7%). All *Salmonella* serotypes were resistant to lincomycin, nalidixic acid and penicilline.In addition, 58.82% of *E. coli* isolates had phenotypic resistance to at least three or more antimicrobials. Our results indicated that the high frequency of tetracycline resistance (68.62%) due to the *tet* (A), *tet* (B), and *tet* (D) genes. This is the first report isolating *S.* Bisberg and determining antibiotic susceptibility of *E.coli* and *Salmonella* isolates from wild birds in Turkey. These results will help providing better understand of the dissemination of antibiotic resistancy in the environment, which can be used to potentially decrease spread through bird migration. Moreover, these results help assess the risk of spread of resistance from wild birds to humans.

Keywords: *E. coli*, *Salmonella*, antibiotic susceptibility, tet genes wild birds

1. Introduction

There are more than 10,000 bird species throughout the world, which migrate between countries and continents (1, 2). Wild birds carry and transmit more than 40 diseases to humans and animals as a result of this movement, including bacterial, viral, parasitic and mycotic diseases (3). Furthermore, there may be an alarming relationship between wild birds and serious ongoing novel coronovirus (CoV) pandemic all over the world (4). Many recent studies have also emphasized that wild birds are the source of pathogens that cause diseases in humans; they can show signs of infection or appear completely healthy as carriers of pathogens (5). Since wild birds are highly mobile, they can carry pathogens long distances during migration, which introduces a risk of spreading disease beyond local outbreaks.

Wildlife plays a critical role as a reservoir for enteric bacterial pathogens and zoonotic diseases. Many wild bird species gravitate towards untreated sewage, garbage, manure, and other sources of enteric pathogens for their nutritional needs. As a result, *Salmonella* spp., which belongs to the

Enterobacteriaceae family, and *Escherichia coli* are common enteric bacteria that are present as potential pathogens in these settings (6-8).

The development and spread of antibiotic resistance throughout the world has been increasing since the early 1960s, which is seen as a major threat to the global public health of wild birds due to their ability to freely travel over long distances during annual migrations (9, 10). Although potentially pathogenic enteric bacteria have been isolated from many wild bird species, recent studies have highlighted that the role of these birds in human and veterinary diseases has been largely under-researched and further work is needed to determine their role in zoonotic transmission (9). Thus, the aim of this study was to investigate the presence of *E.coli* and *Salmonella* spp. in various wild birds, and characterize it phenotypically regarding serovars, tetracycline resistance genes (Tcrs, *tet*) and antimicrobial susceptibility.

2. Materials and Methods

2.1. Sample Collection, Bacterial Isolation and Identification

Intestine and fecal samples were obtained from 82 dead wild birds found in Afyon (38° 45' 24.787'' N 30° 32' 19.334'' E), Denizli (37° 46' 59.9988'' N 29° 5' 40.9740'' E), Uşak (38° 31' 15.59'' N 29° 20' 18.60'' E) and Eskişehir (39° 45' 58.2948'' N 30° 31' 36.1704'' E) provinces of Turkey (Table 1). The samples were cultured on MacConkey agar (Oxoid, UK) and aerobically incubated at 37°C for 24 h. Lactose positive, pink-to-red colonies were selected and assessed for *E. coli* precence using several biochemical tests (catalase, oxidase, indole, urease, motility, methyl red, citrate, and Voges-Proskauer) (11). All strains were maintained at -20°C in Luria-Bertani (LB) medium containing 15% glycerol until tests were performed.

All collected samples were analyzed for *Salmonella* positivity using ISO 6579:2002/Amd 1:2007. Specifically, samples were inoculated in buffered peptone water (BPW) as pre-enrichment medium and then incubated at 37°C for 18-24 h. After incubation, samples were transferred to Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn) and modified semi-solid Rappaport-Vassiliadis (MSRV) medium and enriched for 18-24 h at 37°C and 24 h at 41.5°C, respectively. The cultures obtained were plated onto xylose lysine deoxycholate (XLD) incubated at 37°C, and then examined after 24 h incubation (12). All presumptive *Salmonella* colonies were characterized biochemically (triple sugar iron (TSI), H_2S, gas formation, voges proskauer (VP), urea, lysine decarboxylase, and β-galactosidase tests) by Microgen® GN-ID A sytem (Microgen Bioproducts, UK) (12).

2.2. Serotyping

The serotyping of microbiologically *Salmonella* spp. positive samples were conducted by slide agglutination using polyvalent and monovalent *Salmonella* "O" and "H" antisera according to the Kauffman-White scheme (12). Assessment was conducted at the Ministry of Health, Directorate General of Public Health, Department of Microbiology Reference Laboratories and Biological Products, National Enteric Pathogens Reference Laboratory in Ankara, Republic of Turkey.

2.3. Antimicrobial Susceptibility Testing

An antimicrobial susceptibility test was carried out using the agar disk diffusion method according to the guidelines from the Clinical and Laboratory Standards Institute on Mueller-Hinton agar (Oxoid Ltd, Hampshire, UK) according to the guidelines from Clinical and Laboratory Standards Institute (13). The following antibiotics were selected: ampicillin (10µg; AMP) amoxicillin (25µg; AX), cefotaxime (5µg; CTX), ceftriaxone (30µg; CRO), ciprofloxacin (5µg; CIP), enrofloxacin (5µg; ENR), erythromycin (15µg; E), gentamicin (10µg; CN), florfenicol (30µg; FFC), kanamycin (5µg; K), lincomycin (15µg; MY), nalidixic acid (30µg; NA) neomycin (30µg; N), doxycycline (30µg; DO), oxytetracycline (30µg; OT), tetracycline (10 µg; T), penicillin (10units; P), sulphamethoxazole trimethoprim (25µg; SXT). The results were obtained by measuring the diameter of the growth inhibition zone around the antibiotic disc for each isolated bacterial strain and recorded as sensitive, intermediate or resistant. Isolates displaying resistance to three or more antimicrobial agents were defined as exhibiting multi-drug resistance (MDR) (13).

Table 1. Distribution of samples collected from various wild birds' species

District	Common name	Scientific name	Movements	No. of birds	Samples
Afyon	*Long-legged buzzard*	*Buteo rufinus*	Migratory	10	intestine, gaita
Eskişehir	*Long-legged buzzard*	*Buteo rufinus*		4	intestine, gaita
Afyon	*White stork*	*Ciconia ciconia*	Migratory	10	intestine, gaita
Eskişehir	*White stork*	*Ciconia ciconia*	Migratory	1	intestine, gaita
Afyon	*Common buzzard*	*Buteo buteo*	Migratory	5	intestine, gaita
Eskişehir	*Common buzzard*	*Buteo buteo*	Migratory	3	intestine, gaita
Uşak	*Common buzzard*	*Buteo buteo*	Migratory	1	intestine, gaita
Afyon	*Rock pegeon*	*Columba livia*	Migratory	4	intestine, gaita
Afyon	*Long-eared owl*	*Asio otus*	Migratory	3	intestine, gaita
Eskişehir	*Long-eared owl*	*Asio otus*	Migratory	1	intestine, gaita
Afyon	*Great white pelican*	*Pelecanus onocrotalus*	Migratory	3	intestine, gaita
Uşak	*Great white pelican*	*Pelecanus onocrotalus*	Migratory	1	intestine, gaita
Afyon	*Common kestrel*	*Falco tinnunculus*	Migratory	3	intestine, gaita
Afyon	*Common swift*	*Apus apus*	Migratory	3	intestine, gaita
Afyon	*House sparrow*	*Passer domesticus*	Non-migratory	3	intestine, gaita
Afyon	*Eurasian Jackdaw*	*Corvus monedula*	Migratory	2	intestine, gaita
Afyon	*Common magpie*	*Pica pica*	Migratory	2	intestine, gaita
Afyon	*Mallard*	*Anas platyrhynchos*	Migratory	1	intestine, gaita
Afyon	*Eurasian Woodcock*	*Scolopax rusticola*	Migratory	1	intestine, gaita
Afyon	*Corn Crake*	*Crex crex*	Migratory	1	intestine, gaita
Afyon	*Barn owl*	*Tyto alba*	Migratory	1	intestine, gaita
Afyon	*Little owl*	*Athene noctua*	Migratory	1	intestine, gaita
Afyon	*Grey heron*	*Ardea cinerea*	Migratory	1	intestine, gaita
Afyon	*Western Marsh Harrier*	*Circus aeruginosus*	Migratory	1	intestine, gaita
Afyon	*Common Pochard*	*Aythya ferina*	Migratory	1	intestine, gaita
Afyon	*European honey buzzard*	*Pernis apivorus*	Migratory	1	intestine, gaita
Afyon	*Imperial eagle*	*Aquila heliaca*	Migratory	1	intestine, gaita
Afyon	*Eurasian jay*	*Garrulus glandarius*	Migratory	1	intestine, gaita
Afyon	*Eurasian hoopoe*	*Upupa epops*	Migratory	1	intestine, gaita
Afyon	*Ruddy shelduck*	*Tadorna ferruginea*	Migratory	1	intestine, gaita
Afyon	*Greater Flamingo*	*Phoenicopterus roseus*	Migratory	1	intestine, gaita
Afyon	*Yellow-legged gull*	*Larus michahellis*	Migratory	1	intestine, gaita
Afyon	*Barn swallow*	*Hirundo rustica*	Migratory	1	intestine, gaita
Afyon	*Long-eared Owl*	*Asio otus*	Migratory	1	intestine, gaita
Afyon	*Western jackdaw*	*Coloeus monedula*	Migratory	1	intestine, gaita
Afyon	*Eurasian Bittern*	*Botaurus stellaris*	Migratory	1	intestine, gaita
Afyon	*Great cormorant*	*Phalacrocorax carbo*	Migratory	1	intestine, gaita
Afyon	*European nightjar*	*Caprimulgus europaeus*	Migratory	1	intestine, gaita
Afyon	*Goshawk*	*Accipiter gentilis*	Migratory	1	intestine, gaita
Denizli	*Griffon vulture*	*Gyps fulvus*	Migratory	1	intestine, gaita

2.4. Detection of *tet* Genes

The detailed sequence information of primer sets are listed in table 2 (14-16). DNA extraction were performed according to the instructions of the Gene JET Genomic DNA Purification Kit (Thermo Scientific, USA). DNAs were stored for use as template DNA at -20°C until amplification. Singeleplex PCR assay was carried out for *tet*(W) gene. The protocol was as follows: 25 µl reaction volumes containing 3 µl MgCl (25 mM), 0.5 µl dNTP (10 mM), 10 pmols of primers and 0.2 µl Taq polymerase (5U/µl). PCR amplifications were performed with the following cycling conditions: 3 min at 94°C, followed by 30 cycles of 1 min at 94°C (denaturation) and 1 min at 54°C (primer annealing), 1 min at 72°C (extension), and 7 min at 72°C (final extension). Multiplex PCR was performed for Tcrs groups, Group I; *tet*(B), *tet*(C) and *tet*(D), Group II; (*tet*(A), tet(E) and tet(G), Group III; *tet*(K), *tet*(L), *tet*(M), *tet*(O) and *tet*(S), Group IV; *tet*A(P), *tet*(Q) and *tet*(X). Each multiplexed group's PCR reaction mix concentration and amplification conditions were carried out following the previous research (15).

Table 2. Tetracyline resistance specific primers

Resistance gene	primer sequence 5'-3'	Amplicon size (bp)
tet(A)	GCT ACA TCC TGC TTG CCT TC CAT AGA TCG CCG TGA AGA GG	210
tet(B)	TTG GTT AGG GGC AAG TTT TG GTA ATG GGC CAA TAA CAC CG	659
tet(C)	CTT GAG AGC CTT CAA CCC AG ATG GTC GTC ATC TAC CTG CC	418
tet(D)	AAA CCA TTA CGG CAT TCT GC GAC CGG ATA CAC CAT CCA TC	787
tet(E)	AAA CCA CAT CCT CCA TAC GC AAA TAG GCC ACA ACC GTC AG	278
tet(G)	GCT CGG TGG TAT CTC TGC TC AGC AAC AGA ATC GGG AAC AC	468
tet(G)	CAG CTT TCG GAT TCT TAC GG GAT TGG TGA GGC TCG TTA GC	844
tet(K)	TCG ATA GGA ACA GCA GTA CAG CAG ATC CTA CTC CTT	169
tet(L)	TCG TTA GCG TGC TGT CAT TC GTA TCC CAC CAA TGT AGC CG	267
tet(M)	GTG GAC AAA GGT ACA ACG AG CGG TAA AGT TCG TCA CAC AC	406
tet(O)	AAC TTA GGC ATT CTG GCT CAC TCC CAC TGT TCC ATA TCG TCA	515
tet(S)	CAT AGA CAA GCC GTT GAC C ATG TTT TTG GAA CGC CAG AG	667
tet(P)	CTT GGA TTG CGG AAG AAG AG ATA TGC CCA TTT AAC CAC GC	676
tet(Q)	TTA TAC TTC CTC CGG CAT CG ATC GGT TCG AGA ATG TCC AC	904
tet(X)	CAA TAA TTG GTG GTG GAC CC TTC TTA CCT TGG ACA TCC CG	468
tet(W)	GACAACGAGAACGGACACTATG CGCAATAGCCAGCAATGAACGC	1843

3. Results

3.1. Bacterial Isolation and Identification

A total of 51 *E. coli* strains were isolated from 22 different wild bird species (*Asio otus, Buteo rufinus, Pelecanus onocrotalus, Falco tinnunculus, Ciconia ciconia, Scolopax rusticola, Buteo buteo, Anas platyrhynchos, Crex crex, Tyto alba, Pelecanus onocrotalus, Athene noctua, Ardea cinerea, Accipiter gentilis, Pernis apivorus, Garrulus glandarius, Tadorna ferruginea, Apus apus, Phoenicopterus roseus, Larus michahellis, Columba livia and Phalacrocorax carbo*). In contrast, *Salmonella* spp. isolates were only recovered from intestine and fecal samples of *Asio otus* (long-eared owl) and *Buteo buteo* (common buzzard).

3.2. Serotyping

Two isolates were serotyped; one was *Salmonella enterica* subsp. *enterica* serovar Bispebjerg (*S.* Bispebjerg; common buzzard) and the other was exhibited the common serotype *Salmonella enterica* subsp. *enterica* serovar Kentucky (*S.* Kentucky; long-eared owl).

3.3. Antimicrobial Susceptibility Testing

E. coli isolates had the greatest antimicrobial resistance patterns for lincomysin (100%), penicilline (96.1%), kanamycin (80.4%), tetracycline (68.6%), oxytetracycline (64.7%), and doxycycline (41.2%). *Salmonella* serotypes were resistant to lincomycin, nalidixic acid and penicilline but *S.* Bispebjerg was totally susceptible to nalidixic acid (Table 3). The majority (58.82%) of *E. coli* isolates exhibited phenotypic resistance to at least three or more antimicrobials. The *S.* Kentucky exhibited MDR to lincomycin, penicilline and nalidixic acid (100%) (Table 4).

Table 3. Antibiotic susceptibility and Tcrs results of *E. coli* (n:51) and *Salmonella* (n:2) isolates

Antimicrobials	Isolates	S		I		R		*tet*(A)		*tet*(B)		*tet*(D)	
		n	%	n	%	n	%	n	%	n	%	n	%
AMP	*E. coli*	18	35.3	-	-	23	64.7	-	-	-	-	-	-
	S. Bispebjerg	1	100	-	-	-	-	-	-	-	-	-	-
	S. Kentucky	1	100	-	-	-	-	-	-	-	-	-	-
AX	*E. coli*	18	35.3	2	3.92	21	41.2	-	-	-	-	-	-
	S. Bispebjerg	1	100	-	-	-	-	-	-	-	-	-	-
	S. Kentucky	1	100	-	-	-	-	-	-	-	-	-	-
K	*E. coli*	4	7.8	6	11.8	41	80.4	-	-	-	-	-	-
	S. Bispebjerg	-	-	1	100	-	-	-	-	-	-	-	-
	S. Kentucky	1	100	-	-	-	-	-	-	-	-	-	-
SXT	*E. coli*	26	51	-	-	25	49	-	-	-	-	-	-
	S. Bispebjerg	1	100	-	-	-	-	-	-	-	-	-	-
	S. Kentucky	1	100	-	-	-	-	-	-	-	-	-	-
ENR	*E. coli*	34	66.7	6	11.8	11	21.6	-	-	-	-	-	-
	S. Bispebjerg	1	100	-	-	-	-	-	-	-	-	-	-
	S. Kentucky	-	-	1	-	-	-	-	-	-	-	-	-
P	*E. coli*	2	3.9	-	-	49	96.1	-	-	-	-	-	-
	S. Bispebjerg	-	-	-	-	1	100	-	-	-	-	-	-
	S. Kentucky	-	-	-	-	1	100	-	-	-	-	-	-
CN	*E. coli*	38	74.5	-	-	13	25.5	-	-	-	-	-	-
	S. Bispebjerg	1	100	-	-	-	-	-	-	-	-	-	-
	S. Kentucky	1	100	-	-	-	-	-	-	-	-	-	-
CRO	*E. coli*	36	70.6	4	7.8	11	21.6	-	-	-	-	-	-
	S. Bispebjerg	1	100	-	-	-	-	-	-	-	-	-	-
	S. Kentucky	1	100	-	-	-	-	-	-	-	-	-	-
CIP	*E. coli*	32	62.7	4	7.8	15	29.4	-	-	-	-	-	-
	S. Bispebjerg	1	100	-	-	-	-	-	-	-	-	-	-
	S. Kentucky	1	100	-	-	-	-	-	-	-	-	-	-
	S. Kentucky	1	100	-	-	-	-	-	-	-	-	-	-

Antimicrobials	Isolates	S		I		R		*tet* (A)		*tet* (B)		*tet* (D)	
		n	%	n	%	n	%	n	%	n	%	n	%
NA	*E. coli*	28	54.9	-	-	23	43.1	-	-	-	-	-	-
	S. Bispebjerg	1	100	-	-	-	-	-	-	-	-	-	-
	S. Kentucky	-	-	-	-	1	100	-	-	-	-	-	-
FFC	*E. coli*	30	58.8	-	-	21	41.2	-	-	-	-	-	-
	S. Bispebjerg	1	100	-	-	-	-	-	-	-	-	-	-
	S. Kentucky	1	100	-	-	-	-	-	-	-	-	-	-
CTX	*E. coli*	36	70.6	-	-	15	29.4	-	-	-	-	-	-
	S. Bispebjerg	1	100	-	-	-	-	-	-	-	-	-	-
	S. Kentucky	1	100	-	-	-	-	-	-	-	-	-	-
MY	*E. coli*	-	-	-	-	51	100	-	-	-	-	-	-
	S. Bispebjerg	-	-	-	-	1	100	-	-	-	-	-	-
	S. Kentucky	-	-	-	-	1	100	-	-	-	-	-	-
T	*E. coli*	16	31.4	-	-	35	68.6	8	22.9	5	14.3	5	14.3
	S. Bispebjerg	1	100	-	-	-	-	-	-	-	-	-	-
	S. Kentucky	1	100	-	-	-	-	-	-	-	-	-	-
DO	*E. coli*	30	58.8	-	-	21	41.2	5	23.8	2	9.5	-	-
	S. Bispebjerg	1	100	-	-	-	-	-	-	-	-	-	-
	S. Kentucky	1	100	-	-	-	-	-	-	-	-	-	-
OT	*E. coli*	18	35.3	-	-	33	64.7	6	18.2	3	9.1	1	3.03
	S. Bispebjerg	1	100	-	-	-	-	-	-	-	-	-	-
	S. Kentucky	1	100	-	-	-	-	-	-	-	-	-	-
N	*E. coli*	36	70.6	4	7.84	11	21.6	-	-	-	-	-	-
	S. Bispebjerg	-	-	1	100	-	-	-	-	-	-	-	-
	S. Kentucky	1	100	-	-	-	-	-	-	-	-	-	-
E	*E. coli*	38	74.5	-	-	13	25.5	-	-	-	-	-	-
	S. Bispebjerg	-	-	1	100	-	-	-	-	-	-	-	-
	S. Kentucky	-	-	1	100	-	-	-	-	-	-	-	-

Ampicillin (AMP), Amoxicillin (AX), Cefotaxime (CTX), Ceftriaxone (CRO), Ciprofloxacin (CIP), Enrofloxacin (ENR), Erythromycin (E), Gentamicin (CN), Florfenicol (FFC), Kanamycin (K), Lincomycin (MY), Nalidixic acid (NA), Neomycin (N), Doxycycline (DO), Oxytetracycline (OT), Tetracycline (T), Penicillin (P), Sulphamethoxazole trimethoprim (SXT), Tetracycline resistance (Tcr)

Table 4. Multi-drug resistance profiles of *E.coli* and *Salmonella* isolates

Resistance profiles	Antimicrobials	*E. coli* (n: 30)		*S.* Kentucky (n:1)	
		n	%	n	%
Resistance to ≥3 antimicrobials	MY, NA, P	-	-	1	100
	SXT, MY, NA, P	2	6.7	-	-
	T, MY, P, TE	1	3.3	-	-
	AMP, T, MY, DO	3	10	-	-
Resistance to ≥6 antimicrobials	AX, T, MY, E, P, TE	1	3.3	-	-
	AMP, AX, T, MY, P, TE	5	6	-	-
	AMP, T, MY, DO, P, TE	1	3.3	-	-
	AMP, AX, SXT, CIP, MY, P, TE	1	3.3	-	-
Resistance to ≥ 9 antimicrobials	AMP, AX, SXT, CIP, MY, DO, FFC, NA, P	1	3.3	-	-
	AMP, AX, SXT, T, CTX, MY, FFC, NA, P, TE	1	3.3	-	-
	AMP, AX, SXT, T, CTX, MY, FFC, NA, P, TE, CR	1	3.3	-	-
	AMP, AX, SXT, CIP, T, MY, CN, FFC, NA, K, P, TE	1	3.3	-	-
	AMP, AX, SXT, CIP, T, MY, CN, FFC, NA, ENR, P,TE	1	3.3	-	-
	AMP, AX, T, CTX, MY, CN, E, FFC, NA, K, P, TE	1	3.3	-	-

3.4. Distribution of Antibiotic Resistance Genes

Of the 51 *E.coli* isolates, 35 (68.62%) carried Tcrs genes; 19 (54.3%) with *tet* (A), 10 (28.6%) with *tet* (B) and 6 (17.2%) with *tet* (D). The *tet* (A), *tet* (B), and *tet* (D) genes were identified in isolates resistant to tetracycline (8 (22,9%), 5 (14,3%), and 5 (14,3%)), oxytetracycline (6 (18.2%), 3 (9.1%), and 1 (3.3%)) and doxyxcycline (5 (23.8%) and 2 (9.5%)) respectively. Moreover, none of the isolates resistance to doxycycline were found to possess the *tet* (D) gene.

4. Discussion

In recent years, it has been increasingly interest in wild life and natural hosts for detecting pathogens and antibiotic resistant bacteria. The ability hazard posed by using antibiotic resistant bacterial colonization of wildlife and the following contamination of the surroundings has been strongly recounted (9, 17, 18). It's far been envisioned that the majority of rising infectious diseases in human beings have a flora and fauna reservoir (18) and the potential switch of antibiotic resistant bacteria from wildlife/surroundings to plants, human beings and domestic animals need to now be noted (9, 18). Thus, evidence suggests a positive correlation between the wild life hosts and antibiotic resistant *Enterobacteriaceae* especially *E. coli* and *Salmonella* spp. (17, 19) Transfer of antibiotic-resistant bacteria/genetic elements found in the feces of wild birds known as transmits from wildlife to animals or humans are approved (20, 21). The role of wild birds as reservoir hosts for some zoonotic pathogens within the *Enterobactericeae* family has been previously investigated in many studies all over the world, including Norway (22), Japan (7, 23), Malaysia (24), USA (25), and Egypt (26).

These findings were reflected in our bacteriological analysis; overall prevalence of *E. coli* and *Salmonella* were 62.2% and 2.44% in examined wild birds. Although *E. coli* positive birds were higher than other findings in Egypt, USA, Arabia, Italy, Brasil and Switzerland, lower than Canada (62.7%), Brasil (69.38%) and Trinidad and Tobago (83.8%); *Salmonella* spp. also recovered nearly similar with the previous reports (21, 27-31). In Contrast to *E. coli* isolation, *Salmonella* spp. carriage of migratory or non-migratory wild bird' intestine or fecal shedding is almost 0- <1% (27). Despite the low recovery of *Salmonella* spp., is an evidence of circulation of serovars in the population (32). Interestingly, some studies in which neither *E. coli* nor *Salmonella* spp. have not been isolated were reported (32-34). In the last twenty years, studies demonstratean increase in the prevalence of isolation of the *Salmonella* spp. from wild birds (7, 35).

Nevertheless, it was also reported that climate conditions in particular, might play a role on the isolation rates of migratory birds (36, 37). *E. coli* and *Salmonella* serovars were recovered autumn-winter and summer were expected in this study. The comparable reason of *E. coli* and *Salmonella* spp. prevalence rates may be variations in sampling (e.g. storage conditions of samples), laboratory strategies employed individual studies or species of wild bird examined, localities, season and bird' feeding habits.

The most remarkable part of the resistance of *E. coli* isolates' was lincomysin and penicillin in our study. World Health Organization (WHO) classified tetracyclines, followed by penicillins, and sulfonamides as highly important antimicrobials (38). In this study, *E. coli* isolates were possessed high phenotypic resistance to all tetracyclines as like as other reports (39, 40). Moreover, 64.7% of the isolates were resistant to ampicilline, this case was significantly differ from various reports (41-43) as there were same studies (44). Although extended-spectrum b-lactamase resistance had limited data for wild birds, a high rate of cefotaxime resistance were detected in various countries such as Porto, Portugal contrast to our resistance rate (29.4%) (40, 45). The results for detection of high antibiotic resistance of *E.coli* isolates against to lincomisin, penicilline, kanamycin and tetracyclines were detected from *long-legged buzzard*

(*Buteo rufinus*) following other species as *Great white pelican* (*Pelecanus onocrotalus*), *White stork* (*Ciconia ciconia*) *and Ruddy shelduck* (*Tadorna ferruginea*). It is noteworthy that the highest prevalence of antibiotic-resistant bacteria was found in aquatic birds, therefore *Ruddy shelduck* and *Great white pelican* could be good examples (17, 34).

Our evaluation of the multidrug resistance patterns of the *E. coli* to 58.82%, which was contrast with previous studies showing 1.5-47.4% (28, 31, 39, 46, 47). Moreover, the most prevelant resistances were to ampiciline, lincomycine, tetracycline, oxytetracycline and sulphamethaxazole trimetoprim. Wild birds are less likely to faced with the antimicrobials than domestic ones. Wild birds can become MDR reservoirs by ingesting contaminated food and water in landfills, livestock farms, wastewater treatment facilities, or sewage systems (48). In fact, another scenario that reveals today's reality is the expansion of urban areas and loss of wildlife habitats, thus showing wild birds could reach the contaminated enviroment (49, 50).

In this study, we found the high frequency of tetracycline resistance (68.62%) due to the *tet* (A), *tet* (B), and *tet* (D) genes. The presence of *tet* (A) was the most frequent, followed by *tet* (B) and *tet* (D). According to the our results, *tet* (A), *tet* (B) and *tet* (D) was confered resistance to three tetracycline preparation is approximately 3-20% and the lower detection of the resistant genes could have been due to indefinite phenotypic resistance, lack of gene expression, or other resistance mechanisms was confirmed (51). It was notable that *tet* (A) gene has also been reported to be a common in contrast to other *tet* genes in *E. coli* from wild birds as poultry (14, 16). In addition, prominent wild birds such as common buzzard, flamingo and owl were recorded in detection of *tet* (A) and *tet* (B) genes in consistence with our results (29, 44, 52).

Prevalence studies in different regions of the world (e.g. Argentina, Brasil, United Kingdom, Australia, Spain, Iran, Sweden, United States, Belgium and Italy) over a 40-year period have identified *S.* Typimurium, *S.* Bredeney, *S.* Hadar, *S.* Agona, *S.* Panama, *S.* Virchow, *S.* Enteritidis, and *S.* Newport, *S.* Haifa, *S.* Chester, *S.* Heidelberg, *S.* Infantis, *S.* Kottbus, *S.* Livingstone, *S.* Veneziana, *S.* Muenster (53, 54). *S.* Typimurium, *S.* Enteritidis and *S.* Infantis are remarkable serovars due to zoonotic importance (55). In contrast, we are the first to detect *S.* Kentucky and *S.* Bispebjerg in the long-eared owl and common buzzard from Turkey in this study respectively. Several other studies have investigated the presence of *Salmonella* spp. in various species such as dove, sparrow, Temminck's seedeater, chestnut-capped black-bird and common kestrel (7, 22, 30, 35). We also previously identified *S.* Hessarek from starlings (56). These data represent a potential avian host range, especially for the genus *Salmonella*, which appears limited in Turkey. It is noteworthy, these may be associated to sporadic *Salmonella* infections and mortalities in particularly young wild birds. Concerning the distribution of many serotypes were not represented a host specific in wild birds than livestock and humans (57).

In present study, a significant cases were represented by *S.* Kentucky also referred as MDR from long eared owl was exhibited resistance to lincomycin, penicilline and nalidixic acid (100%) and by *S.* Bispebjerg was the highest frequency of resistance against lincomycin, penicilline. However, serovars were susceptible or intermediately susceptible to 15 out of 18 tested antimicrobials. In addition, none of the *Salmonella* isolates possesed *tet* gene. To our knowledge, in previous studies antimicrobial resistance concerning serovars due to isolation rates were rather limited, compared to *E. coli*. With respect to the overall, *Salmonella* resistance against to antimicrobials in wildbirds in various researches reported such as beta-lactam, penicillin, sulphonamides, aminoglycosid, tetracycline and quinolones (28, 32, 53). The present study provides a prevalence rate of *E. coli* and *Salmonella* isolates from wild birds in Turkey, which enhances our understanding of the local epidemiology of wild life pathogens and antibiotic resistance profiles. This findings focused on antibiotic resistance, which

remains a significant concern for humans and animals interacting directly or indirectly with wild birds. In addition, further researches should be conducted on public health, resistance mechanisms and genetic diversity of pathogens remain regarding the potential for wild birds to act as reservoirs.

Authors' Contribution

O. S. Y., E. H. K. and D. O. planned and designed the study. O. S. Y. and D. O. performed the experiments, and O. S. Y. and D. O. contributed to the analysis and interpretation of data. O. S. Y. drafted the manuscript. All authors read and approved the final manuscript.

Ethics

This study was approved by Republic of Turkey Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks (Protocol no. E-21264211-288.04-892913), Burdur Directorate of Provincial Agriculture and Forestry (Protocol no. E-39637749-325.04.03-802375) and the institutional ethics committee for the local use of animals in experiments (Protocol no. E-93773921-020-20960).

Conflict of Interest

The authors declare that they have no conflict of interest.

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