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RESEARCH ARTICLE

Microbiological Analysis of Gut Flora and Determination of AntibioticResistance from White Storks *(Ciconia Ciconia)* Resting Area during Migration in Türkiye

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Türkiye'de Göç Sırasında Beyaz Leyleklerin *(Ciconia Ciconia)* Dinlenme Alanlarından Toplanan Örneklerle Bağırsak Florasının Mikrobiyolojik Analizi ve Antibiyotik Direncinin Belirlenmesi

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Öz

Amaç: Göçmen kuşlar, rezervuar konakçılar olarak kıtalar arasında viral, bakteriyel ve paraziter hastalıkları yayabilir. Beyaz leylek *(Ciconia Ciconia)* flora bakterilerinin tüm antibiyotiklere duyarlı olması beklenir. Bu çalışma, göç sırasındaki beyaz leyleklerin dışkı örneklerindeki patojenlerin belirlenmesini, bağırsak florasında baskın olan bakterilerin karakterizasyonunu ve antibiyotik dirençliliklerinin belirlenmesini amaçladı.

Gereç ve Yöntem: Leylek dışkıları (n=101) Mart 2022'de göç yolu üzerinde bulunan Konya, Türkiye'de (37°52′22″N 32°29′32″E) toplandı. Numuneler, mezofilik bakteriler ve Gram-negatif bakteriler (*Escherichia coli, Salmonella spp. Enterobacter spp.* ve *Campylobacter spp.*) yönünden bakteriyolojik olarak incelendi. Klasik mikrobiyolojik yöntemler, Gram boyama ve biyokimyasal testler ile identifikasyon yapıldı. İzolatlar VITEK 2 ve polimeraz zincir reaksiyonu (PZR) ile doğrulandı.

Bulgular: *Escherichia coli* (n=101), *Enterobacter cloacae* (n=10), *Hafnia alvei* (n=3), *Campylobacter jejuni* (n=1) ve *Salmonella* Virginia (n=1) tanımlandı. *E. coli* izolatlarının 32'sinin (%31,68) çoklu ilaca dirençli (MDR), 2'sinin (%1,98) yoğun ilaca dirençli (XDR) olduğu ve *E. coli* izolatlarının 33'ünün (%32,67) fenotipik genişlemiş spektrumlu beta-laktamaz (ESBL) pozitif olduğu ayrıca *E. coli* suşlarının 5'nin (%4,95) avian patojen *E. coli* (APEC) olduğu belirlendi. *S.* Virginia izolatının yalnızca ampisilin ve amoksisilin/klavulanata dirençli olduğu belirlendi.

Öneri: Çalışma sonuçlarına göre leyleklerin flora bakterilerinde antibiyotik direncinin boyutunun ciddi olduğu tespit edildi. Bu kuşlar, göç yolundaki kümes hayvanlarını ve süt çiftliklerini kontamine edebilecek patojenleri dışkılarıyla saçabilir. Bu durum insanların bilinçsiz ilaç kullanımının vahşi hayvanlar üzerinde yarattığı kirliliğin göstergesidir.

Anahtar kelimeler: Antibiyotik direnci, Beyaz leylek, *Ciconia ciconia*, Vahşi göçmen kuşlar

Abstract

Aim: As reservoir hosts, migratory birds can spread viral, bacterial, and parasitic diseases between continents. White storks' *(Ciconia Ciconia)* flora bacteria are expected to be sensitive to antibiotics. This study aimed to determine which pathogens were found in the fecal samples of storks, characterize the bacteria that are dominant in the gut flora, and determine antibiotic resistance during migration.

Materials and Methods: Stork's feces (n=101) were collected in March 2022, Konya, Turkiye (37°52′22″N 32°29′32″E) which is on the migration route. Samples were cultured bacteriologically for the identify mesophilic bacteria Gram-negative (*Escherichia coli, Salmonella spp., Enterobacter spp.* and *Campylobacter spp.*). Classical microbiological methods, Gram staining, and biochemical tests were performed. Isolates were confirmed by VITEK 2 and polymerase chain reaction (PCR).

Results: : *Escherichia coli* (n=101), *Enterobacter cloacae* (n=10), *Hafnia alvei* (n=3), *Campylobacter jejuni* (n=1) and *Salmonella* Virginia (n=1) were identified. Some *E. coli* isolates 32 (31.68%) were multidrug-resistant (MDR) and 2 (1.98%) isolates were extensively drug-resistant (XDR), and 33 (32.67%) isolates were determined to be phenotypically Extended Spectrum β-Lactamase (ESBL) positive also some *E. coli* strains 5 (4.95%) were identified as avian pathogenic *E. coli* (APEC). *S.* Virginia isolate was found to be resistant only to ampicillin and amoxicillin/clavulanate.

Conclusion: According to the results of this study, antibiotic resistance in gut flora of white storks is a severe condition. These birds shed pathogens in their feces that can contaminate poultry and dairy farms in the migration route. This situation indicates the pollution caused by people's unconscious drug use effect on wild animals.

Keywords: Antimicrobial resistance, *Ciconia ciconia*, Migration wild birds, White storks

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Introduction

White stork, with its Latin name *Ciconia ciconia*, has been considered a threatened species according to the Red List of the International Union for Conservation of Nature (IUCN) since 2016 (IUCN 2020). As wild migratory birds, white storks feed on pastures, swamps, wetlands, and human landfills. Storks use areas near human habitats for their nests, such as building high-positioned chimneys and electric poles. Through their long-distance movements across continents, they can become vectors of many pathogens (Wu et al 2021). In addition, the stress caused by migration on the birds may cause the collapse of their immune systems and the shedding of pathogens (Foti et al 2011). Migratory birds are vulnerable to virus infections such as influenza (H1N1, H5N1), Newcastle virus, West Nile virus, herpes virus, bacterial pathogens; *Mycobacterium avium*, *Salmonella spp.*, *Anaplasma phagocytophilum*, *Chlamydophila psittaci*, *Borrelia burgdorferi*, *Campylobacter jejuni*, *Escherichia coli* and serve as biological vectors ectoparasite (Smith et al 1996, Horimoto and Kawaoka 2001, Hernandez et al 2003, Reed et al 2003, Hubálek 2004, Szczepańska et al 2015, Bouaziz et al 2018, Zerek et al 2023).

During storks' migration, birds of different species often come together in resting areas, and infections are transferred horizontally (Hubálek 2004). Other features that affect the potency of infection among birds include the diversity of migratory bird populations, flight distance during migration, feeding types, and resting points along the route (van Dongen et al 2013, Stanley et al 2015). Host factors, geographical regions, climatic conditions, and biological factors in the ecosystem also contribute to the spread of pathogens across continents. For this reason, examining and recording bird migrations ensures that bird-borne infections are kept under control and prevents the emergence of new epidemics. This study aimed to detect pathogenic bacteria in white storks' intestinal flora during migration and determine their antibiotic susceptibilities.

Material and Methods

Sample collection

The study was performed during the white stork's immigration season in March 2022. A total of 101 different fresh stool samples were collected in the sterile sample container without stressing the storks in the resting areas on the borders of Konya provinces (37°52′22″N 32°29′32″E), which are on the migration route of white stork in middle Turkiye. Samples were forwarded to the laboratory for microbiological analysis at 4 °C (Figure 1).

Microbiologic analysis of stool samples

For pre-enrichment, one g of feces was incubated in nine mL peptone water (CM1049, Thermo Scientific, UK) for 12 h at 37 °C. Enrichment media were transferred to blood agar to isolate general mesophilic bacteria in the stool. For *E. coli* isolation, 50 µl of pre-enrichment feces broth was passaged on MacConkey agar (MC) (70143, Merck Millipore, GER) (pink colonies), Eosin Methylene Blue agar (EMB) (70186, Merck Millipore, GER) (metallic green colonies), and Rambach agar (1.07500, Merck Millipore, GER) (blue-green colonies), incubated at 37°C for 24 h. For Salmonella isolation, one mL of the enriched sample was transferred on nine mL of Rappaport Vassiliadis Salmonella Enrichment Broth (NCM0103B, Neogen MI, USA) and incubated at 42°C for 24 h. Then, media was transferred with a loop to XLT–4 agar (black colonies) (CM1061, Thermo Scientific, UK) and Rambach agar (red colonies) incubated at 37°C for 48 h. Pre-enrichment cultures were streaked on Campylobacter agar and cultured at 42°C, 10% CO_2 conditions for 48 h. *Campylobacter jejuni* colonies were detected as gray colonies on a black background. All strains were identified by Gram staining and also biochemically confirmed (Three-tube method, Voges-Proskauer (VP) test, Oxidase test) (Lassen 1975, Smith and Hussey 2005, Lehman 2014).

Identification of bacteria with molecular methods and VITEK-2

After all identification tests were completed, suspicious colonies were tested with The Vitek-2 Compact system (VITEK 2 BioMérieux, FR). A Vitek test card (Gram-negative or Gram positive for identification) for biochemical analysis. The card was filled with a suspension of bacteria isolates equivalent to 0.5-McFarland (McFarland DEN, Biosan, LV) standard turbidity, and tests were completed according to instructions.

With the molecular identification, the 16S conserved gene regions of the bacteria were completed according to previous methods (Sayın et al 2016, Sakmanoğlu et al 2021). DNAs of the bacteria were isolated according to DNA purification kit protocol (Promega, WI, USA). Previous studies were followed for primer pairs (Table 1), PCR mixture, and amplification thermal cycling (T100, Bio–Rad, CA, USA) (Rahn et al 1992, Ridell et al 1995, Hoffmann and Roggenkamp 2003, Uslu et al 2024). PCR products and 100 bp DNA ladder were run on

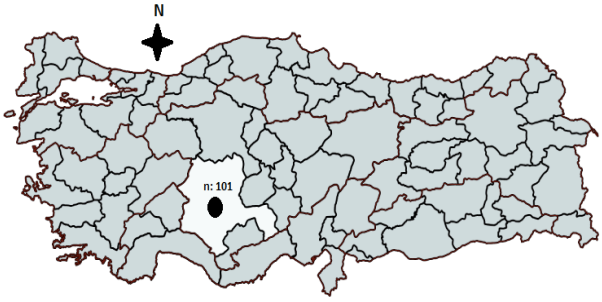

Figure 1. Geographical location of the different white stork nestlings sampling areas in Konya provinces (37°52′22″N 32°29′32″E)

a 1% agarose gel and visualized with a gel imager (212 Pro, Gel-Logic Care Stream Health Inc, NY USA).

Serotyping of Salmonella serovar and Avian Pathogenic Escherichia coli

Avian Pathogenic *E. coli* (APEC) serotyping was performed with O1, O2, O36, and O78 monoclonal antisera (SSI Diagnostica København, DK) as stated in the kit protocol, using the SAT method to form lace in the microplate (Uslu et al 2024). The Salmonella isolate that gave positive results by the VITEK 2 and PCR was serotyped according to the Kauffman-White method (ISO 2017) at the Etlik Central Veterinary Control Institute.

Determination of antibiotic susceptibility

The antibiotic susceptibility tests were carried out as stated in the Clinical and Laboratory Standards Institute (CLSI) guidelines. All strains with McFarland 0.5 standard turbidity were passaged on Mueller Hinton agar (70191, Merck Millipore, GER), and incubated with antibiotic discs (AMP: ampicillin, AMC: amoxicillin/clavulanate, CAZ: ceftazidime, CFX: cefotaxime, TE: tetracycline, IPM: imipenem, SXT: trimethoprim/sulfamethoxazole, CN: gentamicin, CIP: ciprofloxacin) (Oxoid, UK) at 37°C for 12 h (Telli et al 2018). Zone diameters formed around the discs were evaluated as stated in the clinical breakpoint Clinical and Laboratory Standards Institute (CLSI) 2022 data. According to the antimicrobial resistance of *E. coli* isolates, if they were resistant to at least three antimicrobial families, they were classified as multidrug-resistant (MDR), if they were susceptible to only one or two antimicrobial groups, they were classified as extensively drug-resistant (XDR), and if they were resistant to all antimicrobial agents, they were classified as pandrug-resistant (PDR). The phenotypic Extended Spectrum Beta-Lactamase (ESBL) tests were completed using the double disc synergy test, which uses cefotaxime, ceftazidime, and amoxicillin/clavulanate discs (Oxoid, UK) (CLSI 2022). Zone diameters evaluated as mentioned in EUCAST epidemiological cut-off values (ECOFFs) (Kahlmeter and Turnidge 2022). The *E. coli* ATCC 25922 strain was added to the study for antimicrobial test control.

Results

From the microbiological analysis of stool samples taken from 101 white storks, and one to ten presumptive colonies were picked out, 358 bacteria were identified from non-selective and selective media. At least one *E. coli* (101 strains, 100%), was isolated from all 101 fecal samples taken from individual white storks. A total of 101 *E. coli* isolates (100%) were isolated and identified from the stool samples. Additionally, 10 *E. cloacae* isolates (9.9%), 3 *H. alvei* isolates (2.97%), 1 *C. jejuni* isolate (0.99%), and 1 *S.* Virginia isolate (0.99%) were also isolated and identified from the stool samples.

Among the total 101 isolates, 32 (31.68%) of the *E. coli* isolates were MDR and 2 (1.98%) isolates were XDR. And 36 (35.64%) of the total isolates were detected as sensitive to all antimicrobial agents. Of the total *E. coli* strains, 53 (52.4%) were detected resistant to ampicillin, 43 (42.5%) to cefotaxime, and 39 (38.6%) to amoxicillin/clavulanate. Only two of the *E. coli* strains were found to be resistant to gentamicin. All the strains were found to be sensitive to imipenem. Of the 101 isolates, 25.7% were determined to be resistant to ceftazidime and 42.5% to cefotaxime, and 33 (32.67%) of these resistant *E. coli* isolates were determined to be phenotypically ESBL positive.

It was determined that 90% of *E. cloacae* isolates were resistant to ampicillin, 80% of these isolates to amoxicillin/clavulanate, 50% to cefotaxime, 40% to ciprofloxacin, 20% to ceftazidime, and 20% to tetracycline. All Enterobacter strains were sensitive to gentamicin, imipenem, and trimethoprim/sulfamethoxazole. All *H. alvei* and *S.* Virginia isolates were determined to be resistant to ampicillin and amoxicillin/clavulanate. *S.* Virginia strain was detected to be sensitive to all antimicrobial agents except the penicillin group. *C. jejuni* isolate was found to be sensitive to all antimicrobials (Table 2).

As shown in the detailed somatic antigen test results on these 101 *E. coli* strains, O2 somatic antigen was detected in one isolate, O36 in three isolates, and O78 in one isolate, each in a different isolate. These five isolates were evaluated as Avian pathogenic *E. coli* APEC (Table 3).

Discussion

The According to the IUCN 2006 report, it was indicated that there are an estimated 6195 pairs of storks in Turkiye (IUCN 2020). The Sarimazi Adana, Turkiye Bird Observatory report, 83109 storks returning from Turkiye and various European countries between 1-30 September 2022 were reported to have migrated through this region (Arslan et al 2022). Konya province, where the samples were collected in the study, is located on this migration route (Özkazanç and Özay 2019). The sample was collected in March 2022, during the white stork migration from the tropical climate zone to northern countries to spend the summer. It is known that migratory white storks are reservoirs for the intercontinental spread of pathogenic viral diseases, especially West Nile virus, avian influenza and Crimean-Congo hemorrhagic fever (Müller et al 2009, Kaleta and Kummerfeld 2012, Lindeborg et al 2012, Gale and Johnson 2014, Camacho et al 2016).

Table 1. List of primers used to identify bacteria isolated from the gut flora of storks

Gene	Forward/Reverse	Primer sequence	Product	Reference
Salmonella spp. invA	F	GTGAAATTATCGCCACGTTCGGGCAA	284	(Rahn et al 1992)
	R	TCATCGCACCGTCAAAGGAACC		
H. alvei 16S	F	CTGGAACTGAGACACGGTCC	439	(Ridell et al 1995)
	R	GTCAGTCTTTGTCCAGGGGG		
E.cloacae RpoB	F	AACCAGTTCCGCGTTGGCCTGG	1088	(Hoffmann and Roggenkamp 2003)
	R	CCTGAACAACACGCTCGGA		
E. coli fimA	F	TGGTGGGACCGTTCACTTTA	443	(Uslu et al 2024)
	R	AAGGTCGCATCCGCATTAG		

Pathogenic species including avian pathogenic *E. coli*, *S.* Virginia, *C. jejuni, E. cloacae* and *H. alvei* were identified in this study. It is thought that white storks may infect Salmonella because their diet includes creatures that come into contact with sewage, such as mice, frogs and insects (Tsachalidis and Goutner 2002). In addition, due to urbanization, climate change, drought and insufficient food during periods when the young need intense nutrition, storks head to areas where human waste is concentrated, such as garbage dumps (Gilbert et al 2016, Chenchouni 2017). It was stated that *S.* Virginia was first time detected in Turkiye in a study conducted in 1965 (Töreci et al 2013). However, it has not been reported in any living creature in Turkiye since then. Although various Salmonella serovars (*S. Enteritidis*, *S.* Typhimurium, *S.* Chester, *S.* Infantis, *S.* Kentucky, *S.* Abony, *S.* Pomona, *S.* Saintpaul, *S.* Bispebjerg) were isolated in previous studies conducted on white storks, in this study S. Virginia was isolated for the first time from white storks (Höfle et al 2003, Camacho et al 2016, Martín-Maldonado et al 2020, Kandir and Öztürk 2022, Mahmood et al 2022). In this study, only one sample (0.99%) was found positive for Salmonella. Other studies on this subject have stated that salmonella infection in storks varies between 4.4% and 20% (Camacho et al 2016, Martín-Maldonado et al 2020). The *S.* Virginia strain in the study was found to be resistant only to amoxicillin/clavulanate and ampicillin. In another study, the rate of Salmonella serovars resistant to two antimicrobials was found to 13.6 % (Martín-Maldonado et al 2020). Since Salmonella can be transmitted vertically through eggs, it is worrying that mothers can directly infect their offspring and more severe shedding will occur with infected generations (Shaji et al 2023). It should be considered that migratory birds are effective in the local epidemics that will occur due to this strain in the future. It should be considered that the source of serovars isolated from distant countries that have not been encountered before may be wild animals that establish contact in distant countries, such as migrating birds. It is thought that the spread of these factors will increase due to the chronic nature of Salmonella infections and the stress caused by migration on white storks. The fact that these birds make this long journey twice a year, generally migrate between the same countries, rest in certain fixed areas along the migration areas, and come into contact with other birds or animals creates a serious scattering area.

Table 2. Antimicrobial resistance in the *E. coli, E. cloacae, H. alvei, C. jejuni* and *S.* Virgina isolates from white stork feces samples

Antibiotic discs (content)	*E. coli* (n=101)		*E. cloacae* (n=10)		*H. alvei* (n=3)		*C. jejuni* (n=1)		*S.* Virginia (n=1)	
	n	%	n	%	n	%	n	%	n	%
AMC (20/10)	39	38.6	8	80	3	100	0	0.0	1	100
AMP (10)	53	52.4	9	90	3	100	0	0.0	1	100
CTX (30)	43	42.5	5	50	0	0.0	0	0.0	0	0.0
CAZ (30)	26	25.7	2	20	0	0.0	0	0.0	0	0.0
CN (10)	2	1.9	0	0.0	0	0.0	0	0.0	0	0.0
TE (30)	24	23.7	2	20	2	66.6	0	0.0	0	0.0
IPM (10)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
STX (25)	12	11.8	0	0.0	0	0.0	0	0.0	0	0.0
CIP (30)	26	25.7	4	40	0	0.0	0	0.0	0	0.0

AMP: Ampicillin, AMC: Amoxicillin/clavulanate, CAZ: ceftazidime, CTX: cefotaxime, TE: Tetracycline, IPM: Imipenem, SXT: Trimethoprim/sulfamethoxazole, CN: Gentamicin, CIP: Ciprofloxacin

In this study, 4.95% of the *E. coli* strains were identified as APEC. In a study conducted in Spain, 54% of white stork' *E. coli* strains were APEC (O1, O2, O78) (Höfle et al 2020). O1, O2 and O78 somatic antigens are the most common somatic antigens in APEC strains (Mehat et al 2021). Although *E. coli* is a flora bacterium, it is thought that the isolation of APEC *E. coli* is due to the stress caused by factors such as migration-related stress and lack of nutrition on animals. It has been determined that APEC strains can also cause infection in storks. According to our study results, *E. cloacae* was isolated from 9.9% of the white storks and these isolates were detected to be resistant to 80% ampicillin, 90% amoxicillin/clavulanate, 50% cefotaxime and 40% ciprofloxacin, 20% ceftazidime and 20% tetracycline. In a study conducted in Spain on 9 different wild birds, including white storks' samples, *E. kobei* was isolated from white storks and it was reported that it was sensitive to antibiotics (Tardón et al 2021). It has been stated that *E. cloacae* (10%) was isolated from 163 fecal samples from 32 different bird species in Italy and some of these strains were MDR (Russo et al 2022). In the study, a total of 3 *H. alvei* strains were isolated from 101 white stork feces samples. Other study indicated that 2 *H. alvei* isolates were isolated from a total of 183 Enterobacteriaceae isolates in the analysis of the feces of migratory birds in Italy (Foti et al 2011). In a study conducted on seagulls and pigeons in France, it was stated that *H. alvei* was isolated from the gut flora of these birds (Ngaiganam et al 2019). It is also interesting that *C. jejuni* was isolated from the samples in this study. It was reported that 59 strains *Campylobacter spp*. were isolated from 183 fecal samples taken from 5 different bird species in Türkiye (Kürekci et al 2021). In a study conducted in Poland, it was indicated that *C. jejuni* was isolated in 5.3% of 398 fecal samples taken from white stork chicks as a result of 4-year screening. The study also stated that, 42.9% of the isolated *C. jejuni* were detected sensitive to all antibiotics (Szczepańska et al 2015).

In our study, ESBL positive (32.67%), MDR (31.68%) and XDR (1.98%) *E. coli* strains were isolated from white stork feces. Many studies indicated *E. coli* isolates from cattle and sheep in Türkiye were ESBL positive (Pehlivanoglu et al 2016). In the study conducted in 4 bird observatories in Turkiye, 11 out of 82 bird samples were white stork and only *E. coli* was isolated from these birds and those strain resistance to several antibiotic agent (Kandir and Öztürk 2022). Other studies conducted on *E. coli* identified from wild birds in Turkiye, stated that phenotypically and genotypically ESBL positive *E. coli* and MDR *E. coli* (29%) were isolated in various immigratory and wild birds (Ahmed and Gulhan 2024, Halaç et al 2024). White storks like other wild migratory birds are fed with natural resources. They are animals that are unlikely to encounter antimicrobials under natural conditions. Therefore, the antibiotic resistance in question shows that

Table 3. APEC serotypes isolated from *Ciconia ciconia* according to the O somatic antigen

Somatic Antigen	*E. coli* n=101 (100%)
O1	3 (2.97%)
O2	1 (0.99%)
O36	0 (0.0%)
O78	1 (0.99%)
APEC total	5 (4.95%)

resistant enterobacteria created by human beings seriously affect wildlife. It has been stated that some white storks tend to change their feeding and migration habits due to industrialization and urbanization of human beings (Flack et al 2016, Gilbert et al 2016). The detection of antibiotic resistance in strains from wild migratory bird feces isolated from Korea, China, Poland, Algeria, Spain, and Italy reveals the seriousness of the issue (Han et al 2011, Szczepańska et al 2015, Alcalá et al 2016, Bouaziz et al 2018, Höfle et al 2020, Wu et al 2021). According to the results of the study conducted on white storks in Spain, it was reported that 117 *E. coli* isolates isolated were 43% resistant to at least one antibiotic, and 23.1% MDR isolates, 4.5% ESBL-positive *E. coli* (Martínez-Álvarez et al 2023). In a study conducted at the Center of wildlife recovery in Spain, they found that the *E. coli* strain isolated from 4 storks was ESBL positive (Alcalá et al 2016). In the study conducted by Camacho et al (2016) on white storks, it was stated that MDR *E. coli* was identified at a rate of 13%-29%. Höfle et al reported that they isolated ESBL-positive *E. coli* from white storks (Höfle et al 2020). Bouaziz et al study conducted in Algeria that reported one ESBL-positive *E. coli* from white storks (Bouaziz et al 2018). Although developed countries take conscious measures to combat antibiotic resistance, it seems that more global measures are required, rather than on a country basis, with carriers such as migratory birds (Martínez-Álvarez et al 2023). The ecological effects of wild migratory birds such as white storks, which are valuable to the culture of many countries, in terms of pathogenic bacterial diseases are discussed in this study. These birds can contaminate not only migratory routes but also the agricultural lands in the areas where they are staying. Animal feed produced from these agricultural areas poses a significant risk for both poultry and dairy farming. In a comprehensive study examining cephalosporin-resistant *E. coli* strains from feces of white storks feeding on human waste in Spain, the detection of a similar antibiotic resistance mutant in the region 250 km away from the white stork nests and in cattle farms in the region is consistent with the study results (Höfle et al 2020).

Conclusion

Storks and migratory birds are endangered species. These results show the bacterial shedding that migratory birds

can create in the areas where they are found. It is worrying that antibiotic resistance has been detected there in white storks. Instead of looking for all the blame on the movement of migratory birds between several continents, this issue is evidence of how human beings pollute the environment. In this regard, the wildlife institution affiliated with the ministries of agriculture in OIE countries emphasizes that veterinarians and animal breeders should be more careful and sensitive.

Conflict of Interest

The authors did not report any conflict of interest or financial support.

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Author Contributions

Motivation/Concept: AU; Design: AU; Control/Supervision: OE; Data Collection and Processing: ZS, EET, OD; Analysis and Interpretation: AB, AU; Literature Review: AU, ZS; Writing the Article: AU; Critical Review: AU, ZS, OE

Ethical Approval

The Ethical Committee of Selcuk University, Faculty of Veterinary Medicine, Turkiye (No 2024/062) approved this study.