Investigation of beta lactam resistance in Escherichia coli isolated from wild bird feces.

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Abstract

The study's objective was to identify beta-lactamase-resistant Escherichia coli both phenotypically and genotypically in the feces of resident wild birds and passing migrants in the Marmara region. The study involved 272 noninvasively collected fresh fecal samples. The birds of interest were categorized into four groups, assuring that each group comprised 68 samples as follows: Group 1 = resident wild waterbirds; Group 2 = urban resident terrestrial birds; Group 3 = winter migrants; and Group 4 = summer migrants.

Extended-spectrum beta-lactamase (ESBL) and ampicillin Class C (AmpC) beta-lactamase positivity of *E. coli* isolates grown on cefotaxime-supplemented MacConkey agar were assessed by phenotypic screening and confirmation tests in sixty-two (62/272; 22.8%) out of 84 *E. coli* strains, irrespective of group-wise distribution. Fifty of these *E. coli* strains were positive for ESBL, 7 for AmpC, and 5 showed both ESBL and AmpC activities. *E. coli* isolates were detected in fifty-nine (21.7%) of 272 birds, and 57 of these 59 birds were positive for ESBL, six for AmpC, and six for both ESBL and AmpC. Modified Hodge Test revealed no carbapenemase production.

Forty-eight out of 84 *E. coli* isolates grown on cefotaxime-supplemented MacConkey agar were positive for ESBL, three for AmpC, and 5 for both ESBL and AmpC-encoding genes by Polymerase Chain Reaction. The distribution of genes was bla_{CTX-M} (n = 50), bla_{SHV} (n = 2), and bla_{OXA10} (n = 10) for ESBL and bla_{MOX} (n = 6) and bla_{CIT} (n = 2) for AmpC. Carbapenemase genes (bla_{KPC}, bla_{VIM}, bla_{OXA}, bla_{NDM-1}) were undetected.

According to the group and species-wise findings, Group 1, predominantly in seagulls, harbored the highest rates of ESBL- and/or AmpC-producing *E. coli* isolates. Considering that the seagull species that feed on human, animal, and agricultural waste products mainly through garbage dumps are widely distributed in Istanbul, the antimicrobial resistance in *E. coli* strains collected from wild birds is considered to be of human and/or animal origin.

1. Introduction

Doctors can now treat and prevent a wide range of infectious diseases thanks to antimicrobial medications; sadly, antibiotic usage has increased dramatically along with the global population. Antibiotic-resistant bacteria proliferate as a result of this, which is quite concerning. It is evident that resistant bacteria are not restricted by national borders because they are able to spread quickly from person to person, hospital to hospital, and nation to nation. Antibiotic-resistant bacteria can spread so widely that they can infect even healthy individuals in the far-flung Peruvian Amazon (Rolain et al. 2012).

Numerous studies demonstrate that the spread of antibiotic resistance is significantly influenced by wild birds. (Hubálek Z. 2004). Due to its spread, humans, their pets, and agricultural animals are at risk from resistant germs (Reed et al. 2003). There are numerous ways in which birds can develop resistance. Farms, waste sites, landfills, and interactions with other living things are all significant causes of antimicrobial resistance in birds (Zurfluh, K. et. al 2019, Athanasakopoulou, Z. et al. 2022, Dreyer, S. et al. 2022).

Antimicrobial resistance has been found in many animal species, particularly ESBL, when we examine the Turkish portion of the antimicrobial resistance distribution in animals (Kahraman B. et al. 2016, Gümüş B. et al.2017, Sığırcı B et al. 2017). Turkey's geographic location makes it a crucial home for wild birds, particularly migratory ones. Important bird habitats can be found in both isolated rural areas and urban areas. Marmara region, because of its wetlands, it serves as a significant stopover and nesting ground for migrating birds in Turkiye.

The purpose of this study was to identify beta lactam-resistant *E. coli* phenotype and genotype in the feces of wild migratory birds that reside in the Marmara region or go through it for a specific amount of time.

2. Material and Methods

2.1 Study Design

A total of 272 fresh fecal samples noninvasively collected between November 2017 and September 2019 from the Marmara region's resident and passage migrant birds and wild birds that were submitted to the Istanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, the Department of Wildlife Diseases and Ecology by various private veterinary clinics or wildlife rehabilitation centres for therapeutic purposes were evaluated in the study.Four groups were created from the intended wild bird population, and 68 fecal samples were examined in each group (Table 1) in the following ways:

Group 1: resident wild waterbirds of the Marmara region (such as black cormorants, seagulls, and grey herons) inhabiting wetlands near residential districts.

Group 2: resident terrestrial wild birds of the Marmara region (such as doves, pigeons, crows, and sparrows) inhabiting urban areas.

Group 3: winter migrant birds of the Marmara region (periodically stopping over until February).

Group 4: summer migrants of the Marmara region (migrating to the region in the springtime).

Utmost care was taken not to induce acute stress in birds during faeces collection, and the samples were harvested before therapeutic procedures were initiated.

2.2 Bacterial Isolation and Identification

For pre-enrichment, feces were inoculated in Tryptic soy agar and incubated under aerobic conditions for 24 hours at 37°C. (Murk et al. 2009, Overdevest et al. 2011). Then, 1 mL of microbial culture was inoculated onto both cefotaxime (1g/L) supplemented and plain MacConkey agar and incubated at 37°C for 24 h under aerobic conditions. Using standard biochemical testing, isolates exhibiting distinctive morphological characteristics of *E. coli* were identified and validated. Evaluations were performed irrespective of the criterion of a single isolate per specimen.

2.3 Phenotypic detection of ESBL-, AmpC Beta-lactamase, and Carbapenemase-Producing *E. coli*

E. coli isolates identified from cefotaxime-supplemented MacConkey agar were used to investigate the putative presence of ESBL and AmpC beta-lactamases. The specimens of the same bird were categorized in two portions, "a" and "b," to differentiate suspected isolates since morphologically diverse colonies were monitored in some specimens. Phenotypic screening and confirmation tests detected the ESBL positivity. Screening assays were used to determine the sensitivity of *E. coli* isolates to cefpodoxime, ceftazidime, aztreonam, cefotaxime, and ceftriaxone (CLSI 2021). Phenotypic confirmation of ESBL was achieved by the disk diffusion test using cefotaxime (CTX; 30 µg), ceftazidime (CAZ; 30 µg), and these two in combination with clavulanic acid (CAZ/CLA; 30/10 µg) (CLSI 2021).

The putative presence of AmpC beta-lactamase was determined by investigating the cefoxitin and cefepime activities on *E. coli* isolates. Cefoxitin-resistant (≤14 mm) and cefepime-susceptible (≥18 mm) isolates were considered phenotypically positive for AmpC (EFSA 2011).

The Modified Hodge Test determined the putative presence of carbapenemase-producing *E. coli* on the isolates identified from plain MacConkey agar (CLSI 2021).

2.4 Antimicrobial Resistance Genes in *E. coli* Isolates: Molecular Identification

Irrespective of the phenotypic data, PCR was used to look into the potential existence of the beta-lactamase resistance genes AmpC and ESBL (bla_{TEM}, bla_{CTX-M}, bla_{SHV}, bla_{CMY}, bla_{OXA-10}, bla_{PER-2}, bla_{CIT}, bla_{DHA}, bla_{MOX}, bla_{FOX}, bla_{EBR}, and bla_{ACC}) in *E. coli* isolates identified from cefotaxime-supplemented MacConkey agar (Saladin M. et al. 2022, Woodford N. et al. 2006, Arlet G. et al. 1994, Chanawong A. et al. 2000, Perez-Perez FJ. et al. 2002, Vahaboglu H. et al. 1998, Bauernfeind A. et al. 1989) and carbapenemase genes (bla_{KPC}, bla_{VIM}, bla_{OXA}, bla_{NDM-1}) in the isolates identified from plain MacConkey agar (Voets GM. Et al. 2011, Dallenne C. et al. 2010, Manyahi J et. Al 2017).

2.5 Data analysis

For the statistical analysis of the data, SPSS for Windows, Version 13.0 (SPSS Inc. Chicago, USA, released in 2008), was utilized. The relationship between rectal colonization and the presence of the isolate-positivity rate and antibiotic resistance was investigated. When comparing categorical variables, the chi-square test was used; if the unit value was less than 5, Fisher's exact test was used. Statistical significance was established at a p-level of $p<0.05$. To sum up, the data analyses involved the total prevalences of antibiotic resistance, the statistical significance of total prevalences, the prevalences of the groups, and the statistical significance of the prevalences of the groups by pair-wise comparisons.

Table 1. The species and subspecies wise distribution of the collected fecal samples

3. Results

3.1 The Group-wise Distribution of *E. coli* Isolates

Of 272 specimens, 212 and 84 *E. coli* colonies were isolated and identified from plain and cefotaxime-supplemented MacConkey agar, respectively. The group-wise distribution of the identified *E. coli* isolates is summarised in Table 2.

3.2 Data Regarding Phenotypically Detected ESBL, AmpC, and Carbapenemase Activities

Fifty-five (65.47%) of 84 cefotaxime-resistant *E. coli* isolates demonstrated phenotypic ESBL activity, while 12 (14.28%) revealed an AmpC phenotype. Fifty of these isolates were positive for ESBL per se, 7 for AmpC per se, and 5 for comorbid ESBL and AmpC activities. The total number of ESBL- and/or AmpC-positive isolates was 62/272 (22.8%) (Table 2).

In Groups 1, 2, 3, and 4, the distribution of phenotypically ESBL-positive isolates was 32 (66.6%), 13 (65%), 8 (80%), and 2 (33.3%), in that order. Conversely, in Groups 1, 2, and 4, the isolates exhibiting phenotypic AmpC positive were distributed as follows: 9 (18.8%), 1 (5%), and 2 (33.33%). Five *E. coli* isolates exhibited coexistence phenotype of both ESBL and AmpC, with four from Group 1 and one from Group 4.

Modified Hodge Test revealed no carbapenem resistance in any *E. coli* (n=212) isolates grown on plain MacConkey agar.

Table 2. The group-wise distribution of the phenotypically characterized ESBL- and/or AmpC-producing *E. coli* isolates

3.3 Data Regarding the Species-wise Phenotypic Characterization of the Antimicrobial Resistance of *E. coli* Isolates

It was found through phenotypic analysis that 49 out of 272 birds carried isolates of *E. coli* that were resistant to antibiotics. Antimicrobial resistance was associated with 47 cases of ESBL activity per se, 6 cases of AmpC activity per se, and 6 cases of 59 birds with concomitant ESBL and AmpC activities. Thirty-five waterbirds in Group 1 were phenotypically demonstrated to have antimicrobial-resistant *E. coli* isolates. (n=37). The antimicrobial resistance profiles of these 35 birds were associated with ESBL production in 26, with AmpC in 4, and with a coexistence of ESBL and AmpC production in 5 birds. Thirty-four of 35 waterbirds consisted of seagulls (29 Herring Gulls and five little gulls) and one grey heron. In Group 1, 57.6% (34/59) of the fecal samples collected from seagulls were noted to harbour phenotypically resistant *E. coli* isolates.

Antimicrobial-resistant *E. coli* isolates were found phenotypically in 14 terrestrial birds in Group 2. Two doves, nine pigeons, one crow, and one magpie were among the animals whose antimicrobial resistance to E. coli isolates was linked to ESBL in 13 cases and AmpC in one case.

In Group 3, phenotypically antimicrobial-resistant *E. coli* (n=7) were isolated in seven birds, represented by two owlings, two hawks, and three falcons, and the antimicrobial resistance was associated with ESB in all these species.

Three birds represented by one starling, one pygmy cormorant, and one squacco heron in Group 4 were shown to carry phenotypic antimicrobial-resistant *E. coli* isolates (n=3). Among the *E. coli* isolates determined from these wild birds, ESBL was detected in 1, AmpC in 1, and ESBL and AmpC in 1.

3.4 Polymerase Chain Reaction-based data regarding antimicrobial resistance genes of *E. coli* isolates

Regardless of the phenotypic information, beta-lactamase-encoding genes were present in 61 of the 84 *E. coli* isolates cultured on MacConkey agar supplemented with cefotaxime. (51 with ESBL, 2 with SHV, and 8 with AmpC genes). When the phenotypic data was compared with genotypic findings, 41 of 55 phenotypically positive isolates for ESBL were also genotypically positive. Furthermore, 12 of 29 phenotypically negative isolates for ESBL were genotypically positive. Eight of 12 isolates phenotypically positive for AmpC were genotypically positive. On the other hand, none of the isolates phenotypically negative for AmpC was genotypically positive.

3.5 The Distribution of AmpC and ESBL-encoding Genes by Group in *E. coli* Isolates

3.5.1 Group 1: Resident wild waterbirds of the Marmara Region inhabiting wetlands near residential districts

In Group 1, the genotypically detected ESBL- (n=25), SHV- (n=1), and AmpC (n=6) encoding genes of the isolates involved $bla_{CTX\text{-}M}$ (n=25), bla_{SHV} (n=1), bla_{OXA10} (n=6), bla_{MOX} (n=5), and bla_{CIT} (n=1). The ESBL and/or AmpC-producing gene groups were found in 7 isolates (Table 3).

Twenty-eight birds -23 herring gulls, four little gulls, and one grey heron- were shown to be resistant to antibiotics. In twenty-five of 28 birds, the isolates' ESBL encoding genes were part of the $bla_{CTX\text{-}M}$ group, and the most prevalent gene variant was $bla_{CTX\text{-}M\text{-}1}$, with 15 isolates out of 25. Furthermore, six $bla_{OXA\text{-}10}$ and one bla_{SVH} gene were also detected. The $bla_{CTX\text{-}M\text{-}1}$ and $bla_{OXA\text{-}10}$ genes coexisted in three, $bla_{CTX\text{-}M\text{-}1}$, $bla_{OXA\text{-}10}$, and bla_{SVH} in one, and $bla_{CTX\text{-}M}$ and the $bla_{OXA\text{-}10}$ variant in two isolates.

The AmpC-encoding genes were *bla*$_{MOX}$ (n=5) and *bla*$_{CIT}$ (n=1). Two isolates positive for the *bla*$_{MOX}$ gene also exhibited the *bla*$_{CTX-M-1}$ variant in two seagulls, and an isolate cultured from a seagull that harbored the *bla*$_{CIT}$ gene was also positive for *bla*$_{CTX-M-1}$ and *bla*$_{OXA-10}$.

3.5.2 Group 2: (Resident Terrestrial Wild Birds of the Marmara region inhabiting Urban Districts

ESBL (n=14), SHV (n=1), and AmpC (n=1) producing genes were detected in the isolates cultured from Group 2, with the distribution in descending order as *bla*$_{CTX-M}$ (n=15), *bla*$_{SHV}$ (n=1), *bla*$_{OXA10}$ (n=1), and *bla*$_{MOX}$ (n=1).

The number of birds with antimicrobial-resistant *E. coli* isolates was 15, and all isolates possessed the *bla*$_{CTX-M}$ gene group. Seven of 15 isolates also carried the *bla*$_{CTX-M-1}$ variant. Moreover, *bla*$_{CTX-M-1}$, *bla*$_{OXA-10}$, and *bla*$_{SHV}$ coexisted in an isolate cultured from one pigeon. Conversely, an *E. coli* isolate positive for *bla*$_{CTX-M-1}$ that was cultivated from a magpie included the AmpC producing *bla*$_{MOX}$ gene. The genotypically antimicrobial-resistant *E. coli* isolates were most prevalent in pigeons (n=12), followed by one dove, crow, and magpie. One phenotypically negative pigeon was found to be genotypically positive. Moreover, a phenotypically ESBL-negative and AmpC-positive magpie was genotypically determined to harbour both ESBL- and AmpC-encoding genes.

3.5.3 Group 3: Winter Migrant Birds of the Marmara Region

In Group 3, the antimicrobial resistance was genotypically detected in 7 *E. coli* isolates from seven birds, represented by one owling, two falcons, and four hawks. Five of 7 *E. coli* isolates with genotypic ESBL resistance. Four of these five isolates were found to belong to the *bla*$_{CTX-M-1}$ group. Additionally, only one *bla*$_{OXA-10}$ gene was detected in 2 hawks. Neither AmpC activity nor coexisting genes were determined in this group.

3.5.4 Group4: Summer Migrant Birds of the Marmara region

In Group 4, five birds (two starlings, two squacco herons, and one pygmy cormorant) were genotypically determined to show antimicrobial resistance. The ESBL-(n=5) and AmpC- (n=1) encoding genes of the *E. coli* isolates harbored by these birds involved *bla*$_{CTX-M}$ (n=5), *bla*$_{OXA10}$ (n=1), and *bla*$_{CIT}$ (n=1).

The ESBL- and AmpC-encoding genes, *bla*$_{CTX-M}$, *bla*$_{OXA10}$, and *bla*$_{CIT}$, and the ESBL-encoding genes, *bla*$_{CTX-M}$ and *bla*$_{OXA10}$ coexisted in the isolate of one pygmy cormorant and one squacco heron, respectively.

3.6 Genotypic Detection of Carbapenemase Resistance

Carbapenemase resistance genes (*bla*$_{KPC}$, *bla*$_{VIM,}$ *bla*$_{NDM}$, *bla*$_{OXA-48}$) were detected in none of the 212 *E. coli* isolates grown on plain MacConkey agar.

Table 3: Species-wise Antimicrobial Resistance Profiles of the Study's Bird Population

3.7 Statistical Data

Prevalence ratios (PR) and corresponding 95% confidence interval values (95% CI) revealed a phenotypic prevalence of 23.5% and a genotypic prevalence of 21%. When the groups were individually evaluated, phenotypic and genotypic prevalences of antimicrobial resistance were 57.4% and 41.2%, 20.6% and 23.5%, 11.8% and 10.3%, and 4.4% and 8.8% in Group 1, Group 2, Group 3, and Group 4, respectively. Considering the antimicrobial resistance of all study groups, the positivity and negativity rates were 23.5% and %76.5, respectively, with a statistically significant difference ($p<0.001$).

The categorical variability of phenotypic and genotypic positive rates between the groups was compared pairwise, and the results showed that there were statistically significant differences between Group 1 and all other groups. The genotypic difference was statistically significant ($p=0.04$), whereas the phenotypic difference between Groups 2 and 3 was not statistically significant. However, the variations in positivity and negativity rates between Group 3 and Group 4 were statistically inconsequential, but the phenotypic and genotypic differences between Group 2 and Group 4 were statistically significant ($p=0.004$ and $p=0.02$, respectively) (Table 4).

Table 4. Group-wise Comparisons of the Categorical Variable of Phenotypic and Genotypic Positivity

4. Discussion

The emergence of antimicrobial resistance (AMR) due to several factors has become a crucial multifaced issue, adversely impacting humans, animals, and the environment. Several studies demonstrated that the role of wildlife, which is not directly exposed to antibiotics under normal conditions, should be considered in the emergence of AMR. It was previously reported that migratory birds had the potential to spread resistant bacterial strains, threatening human and animal health, to large geographical areas during their annual migration (Hubálek et al., 2004; Reed et al., 2003, Allen et al. 2010).

The exchange of antibiotic-resistant bacterial strains, with varying prevalences of ESBL, among urban resident aquatic or non-aquatic wild birds feeding on anthropogenic sources such as dump sites and wastewater has been well documented in the literature. The researchers stressed that higher prevalences are more likely detected in big, crowded cities, where residential areas with inadequate infrastructure and health care facilities lacking sufficient sanitation intertwine with industrial zones (Nelson et al. 2008, Poeta et al., 2008, Bonnedahl & Järhult, 2014; Borges et al., 2017, Oteo et al., 2018; Islam et al., 2021,Tapia-Arreola, A. K et al. 2022, Freire, S. et al., 2022). Seagulls were mainly shown to be putatively responsible for the potential spread of multidrug-resistant *E. coli* strains, particularly in the coastal settlements, due to their feeding habits through animal and human wastes in garbage dumps (Bonnedahl et al., 2009, Simões, 2010, Poirel et al., 2012 Ahlstrom et al. (2019) Ahlstrom et al. (2021) Zeballos-Gross et al. (2021). In another study, AMR-resistant *E. coli* and AMR-resistant *K. pneumoniae* were isolated from 30.1% (69/229) and 8.73% (20/229), respectively, from seagull faeces collected in city centres and seasonally-active holiday resorts of ten districts of Western Australia's coastline. All of the AMR isolates were found to have originated from samples that were gathered in urban settings, indicating a clear relationship between the proximity of animals to residential areas inhabited by humans and the transmission of AMR bacterial strains among animals. (Mukerji et al. 2021).

The non-aquatic resident birds inhabiting the cities, such as pigeons and crows, were shown to have higher ESBL prevalences than domestic fowl and other birds, and notably, wild pigeons and crows are recognised presumptive reservoirs of multidrug-resistant and ESBL-producing *E. coli*, eliciting the spread of the pathogens in urban areas (Parker et al. 2016,Cunha et al., 2019, Ngaiganam vd., 2019).

In the study, 54.4% (37/68) of cefotaxime-resistant *E. coli* isolated from the waterbirds evaluated in Group 1 revealed phenotypic positivity for ESBL and AmpC, which comprised 51.5% (35/68), represented predominantly by 34 seagulls (29 herring gulls and five little gulls) and a single grey heron, of this group's bird population. That a phenotypic AMR was mainly detected in seagulls in the majority of this species population (34 of 59), which was also compatible with previous studies, is indicative of the possibility that the AMR profile originated in humans or animals, considering the high population of seagulls in the province of Istanbul feeding mostly in garbage dumps, directly consuming human/animal wastes or agricultural waste products.

A prevalence of 20.6% (14/68) for phenotypic ESBL and AmpC positivity in the resident terrestrial birds in Group 2, with a predominance of pigeons (64.3%; 14/68) was suggestive of this species' crucial role in serving as main reservoirs and spreaders of resistant *E. coli* since urban wild birds that are not exposed to antibiotics under normal conditions, yet have close contact with human and animal wastes by various occasions, such as nesting in resting areas provided with contaminated water sources close to the sewage line and feeding on human wastes through garbage dumps and animal manure as agricultural wastes or grassland invertebrates in contaminated soil in the residential areas with vast human and animal population.

Several studies worldwide have suggested that wild winter or summer migratory birds are the reservoirs of ESBL-producing *E. coli*. Furthermore, evidence has shown that migratory wild birds can transmit resistant bacterial strains to humans and domestic animals, or vice versa, during their migrations (Fahim, K. M., et.al 2019, Zurfluh, K., ve ark 2019, Athanasakopoulou, Z. et al. 2022, Fuentes-Castillo, D., et.al 2023).

Herein, we detected a prevalence of 10.3% (7/68) for phenotypic ESBL-associated AMR in Group 3's winter migrants and wild predators, with 11.8% (8/68) phenotypically characterized ESBL-positivity in *E. coli* isolated from this group. On the other hand, the prevalences of phenotypic ESBL associated AMR and ESBL positive *E. coli* isolates were 4.41% (3/68) in summer migrants and small passerines that comprise Group 4. Even though the bird species evaluated in these two groups are considered the potential carriers of ESBL based AML, the relatively lower prevalences than in the other groups are associated with their less contact with humans and animals, thus less access to waste products such as garbage, sewage, and contaminated water and soil, which is compatible with previous studies.

The most prevalent beta-lactamase gene in ESBL and AmpC positive *E. coli* isolates obtained from wild birds was introduced as $bla_{\text{CTX-M}}$ (Zurfluh, K., et al. 2019, Athanasakopoulou, Z. et al. 2022, Dreyer, S. et al., 2022). Even though the prevalences of gene variants of the CTX-M-type enzymes vastly vary, depending on the geographical sites, the most common ESBL gene variant in humans, domestic animals, and resident and migratory wild birds was discovered to be $bla_{\text{CTX-M-1}}$. (Kahraman B. et al. 2016, Gümüş B. et al. 2017, Sığırcı B et al. 2017, Cormier, A. C., et al. 2022), followed in descending order by $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-14}}$. As for Beta-lactamase genes, TEM and SHV were less frequently detected (Stedt J et al. 2015, Mohsin M et al. 2017, Athanasakopoulou, Z. et al. 2022, Luo, Y., et al. 2022). The researchers

have emphasised that plasmid-mediated AmpC Beta-lactamases, apart from ESBL, are of global concern since they generate resistance to cephalosporin antibiotics and Beta-lactamase inhibitors of substantial clinical significance (Park Y.S. et al. 2009, Haenni M. et al. 2022). The *bla*$_{CMY-2}$ variant that ranks among the *bla*$_{CIT}$ group genes was reported to be the most prevalent AmpC Beta-lactamase gene in the wild birds of Europa, mainly central Europa and North America (Athanasakopoulou Z. et al. 2022, Medvecky M. et al. 2022). Resident and migrant gulls have been indicated to metaphorically serve as "ecological sponges" by carrying bacteria strains with AMR of critical health-threatening significance and potentially transmitting them to different environments, rendering a vicious cycle (Fahim K.M. et al. 2019, Zurfluh K. et al. 2019, Athanasakopoulou Z. et al. 2022, Fuents-Castillo D. et al. 2023). The most prevalent ESBL genes were found to be the *bla*$_{CTX-M}$ group and its variant *bla*$_{CTX-M-1}$ in *E. coli* isolates with beta-lactamase activity identified from domestic animals and humans in Turkey. Other *bla*$_{CTX-M}$ gene variants and TEM, SHV, and OXA-10-type variants were also detected (Bonnedahl J. et al. 2014, Day M.J. et al. 2016). The predominance of *bla*$_{CIT}$ group genes, apart from the presence of *bla*$_{MOX}$ type genes, was noted in the genotyping studies carried out with AmpC-producing *E. coli* isolates (Carattoli A. 2008, EFSA 2011, Ewers C. et al. 2012).

The quantity of *E. coli* isolates in our investigation that carried beta-lactamase genes was 32 in 28 (23 herring gulls, four little gulls, one grey heron) birds of Group 1, with the distribution of genes as *bla*$_{CTX-M}$ (n = 25), *bla*$_{SHV}$ (n = 1), *bla*$_{OXA10}$ (n = 6), *bla*$_{MOX}$ (n = 5), and *bla*$_{CIT}$ (n = 1). Out of the 25 isolates, 15 *bla*$_{CTX-M}$ genes were found to belong to the *bla*$_{CTX-M-1}$ variant. The detected genes occurred as a single group of genes, a simultaneously present set of genes, or coexisting ESBL and AmpC genes. All AMR genes were found in seagull species, with a prevalence of 39.7% (27/68), except for ESBL and/or AmpC and one *bla*$_{CTX-M}$ group gene. The most prevalent gene group in gulls was *bla*$_{CTX-M}$ (n = 24), and 14 were in the *bla*$_{CTX-M-1}$ group. No *bla*$_{CTX-M-15}$ gene was detected in any isolate. The distribution of the primary and subgroups of genes in the genotypically ESBL (n = 15) and AmpC (n = 1) positive isolates of Group 2 was *bla*$_{CTX-M}$ (n = 15), *bla*$_{SHV}$ (n = 1), *bla*$_{OXA10}$ (n = 1), and *bla*$_{MOX}$ (n = 1). One *E. coli* isolate showed the coexistence of the ESBL genes (*bla*$_{CTX-M}$, *bla*$_{SHV}$, and *bla*$_{OXA10}$), and one isolate had a combination of ESBL and AmpC gene groups (*bla*$_{CTX-M}$, *bla*$_{MOX}$). The *bla*$_{CTX-M-15}$ gene, common in Europe and Africa, was undetected in any isolate. To sum up, the genotypic findings were consistent with those of previous studies regarding *bla*$_{CTX-M}$ and *bla*$_{CTX-M-1}$; however, they differed due to the absence *bla*$_{CTX-M-15}$.

In a study conducted at Aragorn Wildlife Rehabilitation Center in Spain, with fecal samples of 100 bird species in 15 families, 16 were positive for ESBL. The genotyping revealed the occurrence of 9 *bla*$_{SHV}$, three *bla* $_{CTX-M-1}$, and five *bla*$_{TEM}$ positive strains. In another study conducted with predator birds in Rio de Janeiro, all 41 *E. coli* strains isolated from 14 birds were positive for the *bla*$_{CTX-M}$ gene. Moreover, the *bla*$_{CMY-2}$ gene was found in 2 isolates [51,56]. The phenotypic ESBL resistance was detected in 12 out of 111 resident and migratory birds from Tunisia, and all exhibited the presence of the *bla*$_{CTX-M-15}$ gene [57]. In a similar study conducted in Pakistan, *bla*$_{CTX-M}$ was detected in all 26 ESBL-positive samples isolated from 150 migratory birds, and the presence of the *bla*$_{TEM}$ gene was noted in 19 isolates [46]. Finally, a joint study in Canada and Chile revealed that 195 ESBL-positive isolates were determined in 400 fecal samples collected from migratory Franklin's gulls. The *bla*$_{CTX-M}$ gene was detected in 101 isolates in the Chilean part of the study (Bonnedahl J. et al. 2014).

In our study, the distribution of isolates with genotypically detected ESBL (n = 7) genes was *bla*$_{CTX-M}$ (n = 5) and *bla*$_{OXA10}$ (n = 2) in Group 3, which included winter migrants. Neither the coexistence of genes nor an AmpC encoding gene was detected.

As for Group 4, which included summer migrants, the ESBL (n = 5) and AmpC (n = 1) genes were genotypically detected, and the distribution of main and subgroups of genes was *bla*$_{CTX-M}$ (n = 5), *bla*$_{OXA10}$ (n = 1), and *bla*$_{CIT}$ (n = 1). Furthermore, the coexistence of ESBL and AmpC genes, *bla*$_{CTX-M}$, *bla*$_{OXA10}$, and *bla*$_{CIT}$, was detected in 2 *E. coli* isolates, and the coexistence of the ESBL genes, *bla*$_{CTX-M}$ and *bla*$_{OXA10}$ in a single one. The *bla*$_{CTX-M-15}$ gene, common in Europe and Africa, was found neither in Group 3 nor Group 4. The lower prevalence of *E. coli* isolates with AMR in wild migratory birds that spend a few months in the migration destination than in the resident wild birds of the region was attributed to having less contact with contaminated birds.

Ultimately, it was shown that wild birds living in the Marmara region or using it as a stopover location during migration harbored ESBL and/or AmpC Beta-lactamase-producing *E. coli*. The general data suggests that the resident wild bird population has a higher prevalence of ESBL and/or AmpC beta-lactamase-producing *E. coli* than do migrant birds. This finding can be attributed to the resident birds' increased exposure to humans, animals, and their waste products. Wild urban birds that are thought to be immune-system neutral are frequently in close proximity to people and other animals. Gulls and crows that feed on substantially increasing waste products in the residential districts and pigeons, even though not in direct contact with wastes yet reside in large numbers in contaminated environments, may contribute to spreading infectious agents, becoming a potential source of ESBL genes. Pigeons, mainly long-distance flyers (more than 5 km per day), play an epidemiologically significant role in the widespread spread of antimicrobial-resistant bacteria. Additionally, it was discovered that certain seagull species harbored more antibiotic-resistant *E. coli* when they nested in recreational areas with high human populations and contaminated water sources, particularly those near sewage sites or garbage dumps, than did birds living near clean water

sources. This finding suggests that certain seagull species, acting as reservoirs or carriers of resistant bacteria, are accountable for the inter-ecosystem spread of AMR as a result of migrations from one province to another. On the other hand, given that seagulls primarily eat the wastes of these populations and agriculture, maintaining their crucial role as potential spreaders of antibiotic resistance, the exponential increase in antibiotic use in humans and livestock is another aspect of excessively increased AMR.

Declarations

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Author Contribution

BH - Study conception and design, Sampling, conducting experiments, Writing, AIK - Sampling, conducting experiments, Writing, BÇ- Conducting experiments, Data analysis and interpretation, BBK- Data analysis and interpretation, BDS- Conducting experiments, AFB- Data analysis and interpretation, YÇ - Sampling, conducting experiments, SA- Study conception and design, conducting experiments, Writing. All authors reviewed the manuscript.

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Availability of data and materials

Datasets generated during the current study are available from the corresponding author on request.

Ethical approval

This study was approved by Istanbul University Rectorate Animal Experiments Local Ethics Committee Presidency (Approval no: 483502)

Conflict of Interest

The authors declare no competing interests.

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Tables

Table 1. The species- and subspecies-wise distribution of the collected fecal samples

Groups	Species/Subspecies		Number of birds		Total number
Group 1	Seagull	Little Gull	8	59	68
		Herring Gull	51		
	Grey Heron		3		
	Gaunt		1		
	Bittern		4		
	Water Rail		1		
Group 2	Pigeon		38		68
	Dove		12		
	Crow		12		
	Magpie		6		
Group 3	Falcon		16		68
	Hawk		10		
	European honey buzzard		8		
	Night Peck		6		
	Owl	Tawny Owl	1	14	
		Eared Owl	2		
		Woodcock Owl	5		
		Silver Owl	6		
	Wind-sucker		4		
	Short-toed Eagle		2		
	Owling		5		
	Alpine Swift		2		
	Hobby		1		
Group 4	Starling		24		68
	Pygmy Cormorant		14		
	Stork		5		
	Dalmatian Pelican		6		
	Finch		2		
	Squacco Heron		12		
	Glossy Ibis		4		

Table 2. The group-wise distribution of the phenotypically characterized ESBL and/or AmpC-producing *E. coli* isolates

Groups (n)	CMCA*	ESBL-positive*	AmpC-positive*	ESBL and AmpC-positive*	ESBL-positive per se*	AmpC-positive per se*	ESB-and/or AmpC-positive*
Group 1 n=68	48/68	32/48 (66.7%)	9/48 (18.75%)	4/48 (8.3%)	28/48 (58.3%)	5/48 (10.42%)	37/68 (54.4%)
Group 2 n=68	20/68	13/20 (65%)	1/20 (5%)	-	13/20 (65%)	1/20 (5%)	14/68 (20.6%)
Group n=68	10/68	8/10 (80%)	-	-	8/10 (80%)	-	8/68 (11.8%)
Group n=68	6/68	2/6 (33.3%)	2/6 (33.3%)	1/6 (16.6%)	1/6 (16.6%)	1/6 (16.6%)	3/68 (4.41%)
Total n=272	84/272	55/84 (65.47%)	12/84 (14.28%)	5/84 (5.95%)	50/84 (59.52%)	7/84 (8.33%)	62/272 (22.8%)

CMCA: Cefotaxime-supplemented MacConkey Agar; *number and percentage of isolates

Table 3: Species-wise Antimicrobial Resistance Profiles of the Study's Bird Population

Groups	Bird Species with AMR*	n1	Total no & %	Phenotypic			n2	Total no & %	Genotypic ESBL			Genotypic AmpC		AB	
				ESBL	AMPC	ESBL &AMPC			CTX-M	SHV	OXA-10	CIT	MOX	A	B
1	Herring Gull	29	35 (51.5%)	22	3	4	23	28 (41.7%)	21	1	6	1	4	4	5
	Little Gull	5		3	1	1	4		3	-	-	-	1	-	-
	Grey Heron	1		1	-	-	1		1	-	-	-	-	-	-
2	Pigeon	9	14 (20.6%)	9	-	-	12	15 (22%)	12	1	1	-	-	-	1
	Dove	2		2	-	-	1		1	-	-	-	-	-	-
	Crow	2		2	-	-	1		1	-	-	-	-	-	-
	Magpie	1		-	1	-	1		1	-	-	-	1	1	-
3	Owling	2	7 (10.3%)	2	-	-	1	7 (10.3%)	1	-	-	-	-	-	-
	Hawk	3		3	-	-	4		2	-	2	-	-	-	-
	Falcon	2		2	-	-	2		2	-	-	-	-	-	-
4	Starling	1	3 (4.4%)	1	-	-	2	5 (7.3%)	2	-	-	-	-	-	-
	Pygmy cormorant	1		-	1	-	1		1	-	-	1	-	1	-
	Squacco Heron	1		-	-	1	2		2	-	1	-	-	-	1

*: Antimicrobial resistance; n 1: the number of birds with phenotypic AMR; n2: the number of birds with genotypic AMR

AB: coexistence of ESBL and/or AmpC; A: coexistence of ESBL and AmpC; B: coexistence of ESBL and SHV

Table 4. Group-wise Comparisons of the Categorical Variable of Phenotypic and Genotypic Positivity

	Group 2		Group 3		Group 4	
	Fenotype	**Genotype**	**Fenotype**	**Genotype**	**Fenotype**	**Genotype**
Group 1	p<0.001	p=0.028	p<0.001	p<0.001	p<0.001	p<0.001
Group 2			-	p=0.04	p=0.004	p=0.02
Group 3					-	-