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Original article

Zoonotic risk of *Campylobacter* spp. in urban wild birds: Prevalence and antimicrobial resistance profiles in Istanbul, Türkiye

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Abstract

The aim of this study was to determine the prevalence of thermophilic *Campylobacter* species in urban wild birds living alongside humans in Istanbul, to determine the species distribution of isolated strains, and to characterize their antimicrobial resistance profiles. In this study, 150 fresh fecal samples from wild birds living alongside humans (crows and seagulls living along the coastlines, and pigeons living in tourist areas in the city center) were collected from various regions of Istanbul, Türkiye. For *Campylobacter* isolation, mCCDA agar was inoculated, and suspected isolates identified as *Campylobacter* spp. by biochemical tests were identified by PCR. The phenotypic and genotypic antimicrobial susceptibility profiles of the isolated strains were determined. When fecal samples were examined using conventional methods, *Campylobacter* spp. was isolated from 2/150 (1.33%). As a result of mPCR applied to DNAs obtained directly from the examination samples, 16 *Campylobacter* spp. were found in 15/150 (10%). 14 of these (93.3%) were identified as *C. coli*, and 2 (12.5%) were identified as *C. jejuni*. *Campylobacter* was detected in 11 of 127 pigeons (8.66%), while 10 (7.87%) were identified as *C. coli*, and 2 (1.57%) were identified as *C. jejuni*; *Campylobacter* was detected in 4 of 22 seagulls (18.18%), and all were identified as *C. coli*. When the sample collection regions were compared, the frequency of *Campylobacter* spp. was highest in Beyazıt Square (60%), followed by Küçükçekmece Beach (40%). As a result of antimicrobial susceptibility tests and PCR performed for the presence of tet(O), aphA-3 and gyrA genes, gyrA gene was found in both isolates, while tet(O) and aphA-3 genes were not detected. This study revealed that urban wild bird populations resident in Istanbul are a significant reservoir for Thermophilic *Campylobacter* species and pose a potential public health risk.

Keywords: antimicrobial susceptibility, prevalence, thermophilic *Campylobacter*, wild bird, zoonosis

Introduction

Wild birds are crucial and indispensable components of ecosystems, and by skillfully taking on both predator and prey roles, they play a vital part in maintaining global balance. However, the health of these animals can be compromised by various infections and harmful pathogens, which directly affects their life cycles (Whelan et al. 2008, Ahmed and Gulhan 2022). Among these, *Campylobacter* species stand out as pathogenic agents, particularly those that can cause foodborne illnesses in humans (EFSA and ECDC 2023).

Thermophilic *Campylobacter* species are a notable group of bacteria known for their heat tolerance and their presence in the gastrointestinal tracts of numerous bird species. These bacteria are characterized by their ability to survive and proliferate at high temperatures, with dietary habits and environmental conditions being among the most significant factors influencing their population dynamics (Khan et al. 2013). The environmental conditions of their habitat have a direct effect on their survival. These bacteria, residing commensally in the intestines of wild birds, are also of great importance for ecosystem health. The presence of these bacteria in wild birds has been the subject of numerous studies that have revealed their impact on the birds' feeding habits, breeding season behaviors, and even migration routes. Furthermore, the extensive effects of external factors such as climate change on the global distribution of thermophilic *Campylobacter* and wild bird populations are other important issues that require careful attention (Waldenström et al. 2002).

The investigation of thermophilic *Campylobacter* in wild birds is of great significance. On one hand, it provides critical information for understanding the roles of these bacteria within the ecosystem. On the other hand, it is of utmost importance for identifying bacteria that pose a potential risk to human health, for determining their antibiotic resistance profiles, and for elucidating their role in the global problem of antibiotic resistance (Luangtongkum et al. 2009**)**.

The aim of this study was to isolate thermophilic *Campylobacter* species from urban wild birds living in close proximity to humans in Istanbul, Türkiye. Species which pose a risk of transmission to people, and to determine the antimicrobial resistance of these isolates using both phenotypic and genotypic methods.

Materials and Methods

Sample collection

Between November and December 2022, fecal samples from urban wild birds living in close proximity to humans in Istanbul were collected using a random sampling method. A total of 150 fecal samples were taken from crows and seagulls living along Istanbul's coastlines and from pigeons living in tourist areas in the city center, with the number of samples determined by power analysis (G*Power 3.1.9.7), targeting a statistical power of 80% (1-β = 0.80) to detect a prevalence of 10% with a 95% confidence interval. Based on these parameters, a minimum sample size of 138 was required, and thus a total of 150 samples were collected to increase the statistical power of the study. Freshly voided fecal samples were collected from the ground using sterile swabs. From each sample, two swabs were taken: one for conventional culture and one for direct molecular analysis. Of the urban wild birds from which samples were collected, 127 were pigeons, 22 were seagulls, and 1 was a crow. The samples were collected from various districts on the European side of Istanbul, with 15 from the Florya coast, 15 from the Küçükçekmece coast, 15 from the Beyazıt Square, 16 from in front of the Sultanahmet Mosque, 29 from in front of the Eminönü Spice Bazaar, 36 from in front of the Beylikdüzü metrobus stop, 12 from the Beylikdüzü park, and 12 from the Beylikdüzü coast. Ethic permission was taken from the Animal Use and Care Committee at the Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa on 01.03.2022 with number 2022/10.

Isolation and identification

The collected samples were immediately transported to the Istanbul University-Cerrahpaşa Faculty of Veterinary Medicine, Department of Microbiology laboratory under cold chain conditions. One swab from each sample was used for conventional culture, while the second was stored at -20°C for subsequent molecular analysis.

For conventional isolation, swabs were plated onto mCCDA agar, and plates were incubated at 42°C for 48 hours in a microaerobic atmosphere (85% N2, 10% CO_2, 5% O2) using Campygen gas packs. Suspected colonies (Gram-negative, curved, spiral, or gull-wing-shaped, motile, catalase- and oxidase-positive rods) were considered *Campylobacter* spp.. Isolates were then frozen in MHB containing 25% glycerol and stored at -80°C (Quinn et al. 2011).

DNA extraction

For molecular analysis, DNA was extracted from two sources: the isolated colonies and the original fecal samples. The boiling method was used for DNA extraction from both sources, and the extracted DNA was stored at -20°C (Adıgüzel et al. 2018).

Table 1. Target regions, primer sequences and band lengths used in mPCR.

Target	Gen Region	Primers	Band (bp)
Campylobacter spp. (*16S rRNA*)	MD16S1	ATCTAATGGCTTAACCATTAAAC	857
	MD16S2	GGACGGTAACTAGTTTAGTATT	
C. jejuni (mapA)	MDmapA1	CTATTTTATTTTTGAGTGCTTGTG	589
	MDmapA2	GCTTTATTTGCCATTTGTTTTATTA	
C. coli (ceuE)	COL3	AATTGAAAATTGCTCCAACTATG	462
	MDCOL2	TGATTTTATTATTTGTAGCAGCG	
C. lari (glyA)	CLR	CAAGTCTTTGTGAAATCCAAC	560
	CLF	ATTTAGAGTGCTCACCCGAAG	

Amplification

The confirmation and species-level identification for *Campylobacter* spp. (16S rRNA; 23), *C. jejuni* (mapA gene), and *C. coli* (ceuE gene) was performed by multiplex polymerase chain reaction (mPCR) from both the cultured isolates and the direct fecal samples as described by Adıgüzel et al. (2018). PCR mixture contained 12.5 µL of PCR buffer (contained 0.05 U/µl Taq DNA polymerase, reaction solution, 4Mm MgCl2, 0.4 mM dNTP; Thermoscientific), 1 µL of forward and reverse primers (2.5 pmol/µL MD16S1/S2, 10 pmol/µL MDmapA1/A2, and 10 pmol/µL COL3/MDCOL2), 2 µL of target DNA, and up to 25 µL of distilled water. Amplification was performed in a thermal cycler (Himedia, Sacon) with 35 cycles of 95°C for 60 s as initial denaturation, 95°C for 15 s as denaturation, 59°C for 60 s as the annealing step, 72°C for 90 s as extension, and 3 min as the final extension step at 72°C. The products obtained after PCR were subjected to electrophoresis at 200 V in 1% agarose gel for 30 min and were stained with safe-red (0.5 µg/mL) (26). *C. jejuni* C718 and *C. coli* my78 were used as positive control strains. Target regions, primer sequences and band lengths used in mPCR are given in Table 1.

Antimicrobial susceptibility testing

Antimicrobial susceptibility profiles of the isolates were determined phenotypically using the microbroth dilution method with a Sensititre EU Surveillance *Campylobacter* CAMPY2 Plate (Thermo Scientific) (Mencía-Gutiérrez et al. 2021). For the genotypic determination, the isolates were examined by PCR for the presence of the *tet(O)* gene to determine tetracycline resistance, the *aphA-3* gene for gentamicin resistance, and the *gyrA* gene to determine ciprofloxacin resistance (Adıgüzel et al. 2018).

Statistical analysis

For statistical analysis, SPSS version 13.0 was utilized. The Chi-squared test was applied to compare positivity rates across different animal species, bacterial species, and geographical locations. Differences were considered significant at $p<0.05$.

Results

Campylobacter spp. was isolated from 2 (1.33%) of the 150 fecal samples collected from pigeons, seagulls, and a crow residing in close contact with humans by conventional methods. To confirm the 2 suspected *Campylobacter* spp. isolates, identify them at the species level, and also to investigate the presence of *Campylobacter* from 150 fecal samples using direct molecular methods, mPCR was performed. The two isolates identified as *Campylobacter* spp. by conventional methods were confirmed by a band at 857bp. These two isolates were identified as *C. coli* by forming a band at 462bp.

As a result of mPCR applied to the DNA directly obtained from the sample, 15 of the 150 samples were identified as *Campylobacter* spp. by forming a band at 857bp. Of these, 14 (9.33%) samples with a band at 462bp were identified as *C. coli*, and 2 (1.33%) samples with a band at 589bp were identified as *C. jejuni*. One sample provided a simultaneous identification of both *C. coli* and *C. jejuni*. No evidence of *C. lari* was found in any of the samples.

As a result of the study, a total of 16 *Campylobacter* strains were identified from 15 of the 150 (10%) urban wild birds in Istanbul using mPCR. Of these, 14 (87,5%) were identified as *C. coli* and 2 (12.5%) as *C. jejuni*. *C. coli* was detected in 10 (7.87%) of the 127 pigeon samples, and *C. jejuni* was detected in 2 (1.57%) of the pigeon samples. A mixed infection of both *C. coli* and *C. jejuni* was detected in one of the pigeon samples. In the 22 seagull samples, *C. coli* was detected in

Table 2. The distribution of *Campylobacter* isolates by animal species.

Species	Number	No of *Campylobacter* spp. (+) by culture / (%)	No of *Campylobacter* spp. (+) by PCR / (%)	*C.coli*	*C.jejuni*	*C.lari*
Pigeon*	127	-	11 (8.66%)	10 (7.87%)	2 (1.57%)	-
Seagull	22	2 (9.1%)	4 (18.18%)	4 (18.18%)	-	-
Crow	1	-	-	-	-	-

* Both *C. coli* and *C. jejuni* were isolated from one pigeon sample

4 (18.18%). No *Campylobacter* positivity was found in the crow sample. The two isolates obtained by the conventional culture, both from seagull samples, were identified as *C. coli*, confirming the molecular results for those specific samples. When comparing the collection areas, the frequency of *Campylobacter* spp. was highest in Beyazıt Square (60%), followed by Küçükçekmece Coast (40%). No positivity was found in other areas. The distribution of the animals from which *Campylobacter* was isolated, by species, is given in Table 2.

In the phenotypic determination of the antimicrobial susceptibility profiles of the isolates the 2 isolates tested using the Sensititre EU Surveillance *Campylobacter* CAMPY2 Plate were determined to susceptible to all antibiotics.

In the genotypic determination of the antimicrobial susceptibility profiles, they were examined by PCR for the presence of the *tet(O)* gene for tetracycline resistance, the *aphA-3* gene for gentamicin resistance, and the *gyrA* gene for ciprofloxacin resistance. The *gyrA* gene was identified only in two isolates of the 16, whereas the *tet(O)* and *aphA-3* genes were not detected.

Discussion

The high degree of interaction between wild birds, domestic animals, and humans in urban areas underscores the critical importance of investigating these populations for zoonotic pathogens such as *Campylobacter* species. This study aimed to determine the prevalence of thermophilic *Campylobacter* species and their antimicrobial susceptibility profiles in the urban wild bird population of Istanbul. The findings provide crucial data on the role of wild birds in the carriage of *Campylobacter* spp. within urban ecosystems and their potential implications for public health.

In this study, a *Campylobacter* spp. prevalence of 10% was detected in urban wild bird populations living in close contact to humans in Istanbul. This rate is consistent with the wide range of prevalence values reported in the literature from Europe and North America, which vary between 0% and 70%. Such a broad range indicates that numerous variables, including sampling methodology, geographical location, seasonal factors, bird species, and laboratory techniques, can influence the reported prevalence (Abdullahpour et al. 2015, Ramonaite et al. 2015, Du et al. 2019). For instance, while Ramonaite et al. (2015) reported a high prevalence of 36.2% in urban birds in Lithuania, another study in Sweden found a much lower rate of 5.7% (Waldenström et al. 2002). Our study revealed a stark contrast in detection rates between conventional culture (1.33%) and direct mPCR (10%), a critical finding that highlights the superior sensitivity of molecular techniques for detecting *Campylobacter* in environmental samples. This discrepancy is likely attributable to the presence of bacteria in a 'viable but non-culturable' (VBNC) state, a phenomenon well-documented for *Campylobacter* species. While metabolically active, VBNC bacteria do not grow on standard laboratory media, often as a response to environmental stressors. The ability of mPCR to detect the DNA of these VBNC forms, even when they are not culturable, provides a more accurate representation of the true prevalence within the sampled population (Khan et al. 2013). This finding underscores the importance of integrating molecular methods into routine surveillance programs, particularly when investigating pathogens in complex environmental matrices.

Our findings also revealed a higher prevalence of *Campylobacter* in seagulls compared to pigeons (18.18% versus 8.66%). This is consistent with reports from Spain in which *Campylobacter* spp. was detected in gull chicks, with colony-level prevalence's ranging from 2.4% to 18.67% (Ramos et al., 2010) but differs from other studies reporting lower rates (approximately 1%) (Antilles et al. 2021). The higher prevalence in seagulls can be linked to their feeding habits and habitats, as they frequently forage in high-risk areas such as garbage dumps and sewage discharge points, which are prone to *Campylobacter* contamination (Lu et al. 2011). The prevalence in pigeons (8.66%) was similar to rates found in other studies on urban pigeons, such as the 9.1% reported in Montreal (Gabriel-Rivet et al. 2016). These differences in prevalence across species can be attributed to variations in their ecological niches, immune systems, and frequency of pathogen exposure.

Regarding species distribution, our study found that

C. coli was significantly more dominant than *C. jejuni*, identified in 93.3% and 12.5% of the positive samples, respectively. This points to the existence of a different ecology in wild bird populations, contrary to the general perception that *C. jejuni* is the most common species in human campylobacteriosis cases (Mencia-Gutiérrez et al. 2024). This suggests that urban wild birds in Istanbul may serve as a significant local reservoir for *C. coli*, a species commonly associated with farm animals like pigs and poultry (Waldenström et al. 2002, Russo et al. 2021). The high prevalence of *C. coli* in these birds could be linked to their scavenging habits in urban environments, where they may come into contact with contaminated food waste or other sources from domestic animals. Consequently, wild birds likely play a role in the circulation of *C. coli* within the urban ecosystem, which poses a unique and often overlooked public health risk. This highlights the need to reconsider the epidemiological role of different *Campylobacter* species in urban wildlife and underscores the importance of regional surveillance programs to track these distinct transmission patterns. Notably, no *C. lari* was detected in any of the samples, a species typically associated with waterfowl populations (Man 2011, Antilles et al. 2021). This absence suggests that the resident bird populations or the environmental conditions in the sampled areas of Istanbul may not be conducive to the proliferation of *C. lari*. While the lack of this species indicates a low risk of transmission from this source to humans, further research is needed for a more comprehensive understanding of regional epidemiology.

The antimicrobial susceptibility profiles of the isolates were determined using both phenotypic and genotypic methods. Phenotypic tests showed that the limited number of isolates (2 isolates) were susceptible to all antibiotics on the panel. This is a positive finding, suggesting that the effectiveness of current antibiotics for treating *Campylobacter* infections in animal populations, and potentially in humans, may still be preserved in the region (EFSA and ECDC 2023). However, this finding cannot be generalized without testing a larger number of samples. The literature contains numerous reports of increasing resistance rates, particularly to clinically important antibiotics such as fluoroquinolones and macrolides (Luangtongkum et al. 2009), which underscores the global importance of monitoring antimicrobial resistance. Genotypic analysis, which provides insight into the underlying resistance mechanisms, revealed the presence of the *gyrA* gene, which is associated with fluoroquinolone resistance, in both isolates. In contrast, the *tet(O)* gene for tetracycline resistance and the *aphA-3* gene for gentamicin resistance were not detected. The presence of the *gyrA* gene is a critical finding, as it indicates a potential for the rapid development of resistance under selective pressure, even if the isolates are currently phenotypically susceptible (Piddock et al. 2001). This serves as an early warning sign for future resistance issues.

In addition to the zoonotic transmission risk, our study also sheds light on the role of urban wild birds as reservoirs in urban environments and the variation in prevalence across different bird species and geographical locations. The finding of elevated prevalence in areas with significant human activity, such as the Beyazıt Square and the Küçükçekmece Coast, highlights the critical influence of environmental factors and human-animal interactions on pathogen dissemination. These areas, characterized by the concentration of food waste and wastewater discharge, likely increase birds' access to contaminated sources (Obiri-Danso et al. 2001). These results underscore that the role of urban wild birds in *Campylobacter* epidemiology and the potential for zoonotic transmission should be considered a significant factor. Consequently, continuous and comprehensive surveillance studies of urban wildlife are essential for managing potential zoonotic risks and for a more detailed understanding of transmission chains (Dudzic et al. 2016).

In conclusion, this study confirms that urban wild birds in Istanbul, particularly seagulls, serve as a significant reservoir for thermophilic *Campylobacter* species, with a notable dominance of *C. coli*. The higher prevalence observed in areas of intense human activity and waste disposal underscores the critical link between urban environments and pathogen dissemination. While the phenotypic susceptibility of the isolates is a positive finding, the molecular detection of the *gyrA* resistance gene in these strains highlights a potential for rapid resistance emergence, serving as an early warning for future public health concerns. These findings emphasize the necessity of integrating molecular methods into routine surveillance programs. We recommend the implementation of continuous and long-term surveillance studies, ideally at a national level, to monitor the epidemiology of *Campylobacter* in urban wildlife and to better understand the complex dynamics of zoonotic transmission chains.

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Author Declarations

Ethics approval

The approval was taken from the Animal Use and Care Committee at the Faculty of Veterinary Medicine, Istanbul University- Cerrahpaşa (Eth No: 2022/10-01.03.2022).

Use of generative artificial intelligence

Generative artificial intelligence was used for a literature review.

Conflict of interest

There are no conflicts of interest to declare.

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