



Occurrence and antibiotic sensitivity profile of thermophilic *Campylobacter* spp. isolated from soils associated with wild, captive and pet birds[#]

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Abstract

Campylobacter spp. are among the leading causes of foodborne diarrhoeal diseases worldwide, posing a growing public health concern due to emerging antimicrobial resistance. This study investigated the occurrence and antibiotic resistance profile of *Campylobacter* spp. isolates in soil from bird (wild, captive and pet)-associated environments across three districts of Kerala (Thrissur, Kottayam, and Thiruvananthapuram). A total of 60 soil samples were collected from crop fields, nesting sites, zoo enclosures and pet bird cages. Conventional plating for *Campylobacter* spp. was performed using modified Charcoal Cefoperazone Deoxycholate agar, followed by biochemical and molecular confirmation through multiplex PCR. Physicochemical parameters (pH, electrical conductivity and organic carbon) were also analysed to evaluate their association with *Campylobacter* occurrence. *Campylobacter* spp. were detected in 8.3 per cent of 60 soil samples, with the highest occurrence in Thrissur (15% of 20 samples), followed by Kottayam (10% of 20 samples) and absent in Thiruvananthapuram. *Campylobacter jejuni* was the predominant species identified, while *C. coli* was detected in mixed infections. Soils positive for the organism exhibited slightly higher pH (5.9-6.7) and significantly lower electrical conductivity (11-70 $\mu\text{S}/\text{cm}$) compared to *Campylobacter* negative soils. Antibiotic susceptibility testing revealed that all the isolates remained sensitive to macrolides, fluoroquinolones, aminoglycosides and carbapenems. The MAR index ranged from 0.19 to 0.22, indicating moderate antibiotic exposure. These findings highlight soil as a potential environmental reservoir for *Campylobacter*, emphasising the need for routine soil-based surveillance and integrated One Health monitoring of antimicrobial resistance.

Keywords: *Campylobacter*, soil, bird-associated environments, antimicrobial resistance, Kerala, One Health

Foodborne diseases remain a significant public health concern globally, causing millions of illnesses and deaths each year (WHO, 2024). Among the major bacterial agents, *Campylobacter* spp. are the leading causes of foodborne diarrhoeal illness, responsible for an estimated 96 million cases annually. The predominant species, *C. jejuni* and *C. coli*, typically cause self-limiting gastroenteritis but can occasionally result in severe complications such as Guillain-Barré

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syndrome, reactive arthritis and irritable bowel syndrome (CDC, 2024). Although poultry is considered the principal source of human infection, *Campylobacter* is also widely distributed in the environment. Birds excrete large numbers of bacteria through their droppings, which can contaminate soil and water. Under cool, moist and shaded conditions, these bacteria can persist for prolonged periods, making soil a potential environmental reservoir that contributes to the transmission of *Campylobacter* to animals and humans.

Kerala's tropical monsoon climate, abundant wetlands, and its location along the Central Asian Flyway make it a haven for migratory, resident and captive birds. Frequent interactions between these bird populations and humans in crop fields, nesting areas, zoos and pet stations create potential routes for pathogen transmission. The region's moist, organic-rich, and often acidic soils, typical of its lateritic landscape, provide favourable conditions for the survival of *Campylobacter*. The bacterium's ability to enter a viable but non-culturable (VBNC) state further enhances its persistence under adverse environmental conditions (Rollins & Colwell, 1986). Consequently, conventional culture-based detection methods often underestimate its presence, making molecular approaches such as polymerase chain reaction (PCR) essential for accurate identification from soil and other environmental sources. Environmental *Campylobacter* isolates have also demonstrated a greater tendency to acquire antimicrobial resistance through horizontal gene transfer that enable exchange of resistance determinants between co-existing bacterial species.

In recent years, antimicrobial resistance (AMR) in *Campylobacter* has emerged as a major concern. The indiscriminate use of antibiotics in both human and veterinary medicine has contributed to the spread of resistant strains, particularly to fluoroquinolones and macrolides, which are critical for treating severe human infections (WHO, 2020). The detection of resistant *Campylobacter* in environmental sources such as soil and water highlight the need for a One Health approach that considers the interconnectedness of humans, animals and the environment.

Despite the recognized importance of *Campylobacter* as a zoonotic pathogen, information from India on its occurrence and antibiotic resistance in environmental samples remains scarce. Understanding the distribution and resistance profile of *Campylobacter* in soil from bird-associated habitats is therefore crucial for assessing its ecological role and public health impact. Hence, the present study was undertaken to determine the occurrence of *Campylobacter* spp. in soil samples collected from bird-associated environments across three districts of Kerala and to assess their antibiotic resistance profiles.

Materials and methods

Study area and sample collection

Soil samples were collected from different bird-associated environments across three districts of Kerala (Thrissur, Kottayam and Thiruvananthapuram), with 20 samples from each district. The sampling sites represented diverse habitats frequented by birds, including crop fields and riverbanks visited by migratory species, nesting areas of urban birds, pet bird cages and zoo enclosures housing captive wild birds. From each site, about 250 grams of topsoil were aseptically collected from a depth of 5-10 cm, ensuring that the samples were taken at least 30-40 cm away from visible bird droppings to avoid direct faecal contamination. The samples were placed in sterile polythene bags using clean scoops, and both scoops and gloves were changed between each collection to prevent cross-contamination (Xu et al., 2021). Immediately after collection, the samples were transported under chilled conditions in insulated containers and brought to the Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Mannuthy, for further processing.

Isolation and identification of Campylobacter spp.

Isolation of *Campylobacter* spp. from soil samples was carried out following the procedures recommended by Stern et al. (2001) and the OIE Terrestrial Manual (2017), with slight modifications to suit environmental samples. About 25 grams of each soil sample were aseptically transferred to 225 mL of modified Charcoal Cefoperazone Deoxycholate (mCCD) broth supplemented with CCDA selective supplement (FD 135). The samples were incubated under microaerophilic conditions (7% CO₂ and 5% O₂) at 42°C for 48h to favour the growth of thermophilic *Campylobacter* species. Following enrichment, a loopful of the broth culture was streaked onto Blood-Free *Campylobacter* Selectivity Agar (mCCDA) supplemented with CAT selective supplement (FD 145), *Campylobacter* Supplement V (FD 067) and Polymyxin B selective supplement (FD 003). The plates were incubated under the same microaerophilic conditions at 42°C for 48h. Colonies showing characteristic *Campylobacter* morphology (greyish, flat, moist and spreading with a metallic sheen) were selected for further examination. Presumptive colonies were subjected to Gram's staining and biochemical tests, including oxidase, catalase, hippurate hydrolysis and indoxyl acetate hydrolysis, for preliminary species identification as described by Tenover & Fennell (1992).

Molecular confirmation of Campylobacter spp.

All presumptive *Campylobacter* isolates and 48h broth-enriched samples were subjected to multiplex polymerase chain reaction (mPCR) for molecular

confirmation. The assay targeted the *Campylobacter* genus-specific *16S* rRNA gene, the *mapA* gene specific to *C. jejuni* and the *ceuE* gene specific to *C. coli*, as described by Linton et al. (1996) and Denis et al. (1999), respectively. In addition, the *cadF* gene, a conserved virulence-associated gene responsible for host cell adherence, was also targeted in confirming the isolates (Bang et al., 2003). The primers used for identification are summarised in Table 1.

The DNA was extracted from both broth cultures and isolates by the boiling and snap-chilling method (Englen & Kelley, 2000). Briefly, bacterial pellets were suspended in sterile nuclease-free water, heated in a boiling water bath for 10 min, and rapidly cooled on ice to lyse the cells. The supernatant was collected and used as the DNA template for PCR amplification. The PCR reactions were performed in a 30 μ L reaction mixture containing 10 \times PCR buffer, MgCl₂, dNTPs, specific forward and reverse primers, Taq DNA polymerase and 5 μ L of extracted DNA (Table 2). Amplification was carried out in a thermal cycler (Bio-Rad, USA) with the following cycling conditions: initial

denaturation at 95°C for 10 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 51.8°C for 1 minute, extension at 72°C for 1 minute and a final extension at 72°C for 10 minutes. The amplified products were resolved on a 1.5 per cent agarose gel prepared in 1 \times TAE buffer and visualised under UV illumination after staining with ethidium bromide. The presence of characteristic amplicons of 816 bp (*16S* rRNA), 589 bp (*mapA*), 462 bp (*ceuE*) and 400 bp (*cadF*) confirmed the isolates.

Physicochemical parameters of soil

The physicochemical characteristics of the soil samples, including pH, electrical conductivity (EC) and organic carbon (OC), were analysed to understand their association with the presence of *Campylobacter* spp. The analyses were carried out at the Radiotracer Laboratory, College of Horticulture, Kerala Agricultural University, Thrissur, using standard laboratory procedures. These parameters were compared between *Campylobacter* positive and negative soil samples to assess their possible

Table 1. Primers used for the identification of *Campylobacter* spp.

Gene	Primer	Length	Primer sequence	Size (bp)	Ref.
<i>16S</i> rRNA	F	19	5'-GGATGACAC TTTTCGGAGC-3'	816	Linton et al., 1996
	R	18	5'-CATTGTAGC ACGTGTGTC-3'		
<i>cadF</i>	F	20	5'-TTGAAGGTAA TTTAGATATG-3'	400	Bang et al., 2003
	R	20	5'-CTAATACCTA AAGTTGAAAC-3'		
<i>mapA</i>	F	24	5'-CTATTTATTTT TGAGTGCTTGTG-3'	589	Denis et al., 1999
	R	25	5'-GCTTTATTTGCC ATTTGTTTTATTA-3'		
<i>ceuE</i>	F	23	5'-AATTGAAAATT GCTCCAACCTATG-3'	462	Denis et al., 1999
	R	23	5'-TGATTTTATTA TTTGTAGCAGCG-3'		

Table 2. Components of multiplex PCR mixture

Sl. No	Name of the reagent	Stock Concentration	Quantity (μ L)
1	Template DNA	50ng/ μ L	5.00
2	10X PCR buffer	200mM	3.00
3	MgCl ₂	25mM	2.00
4	Taq DNA polymerase	5Units/ μ L	0.75
5	dNTP Mix	2mM each	2.50
6	Forward primer of <i>16S</i> rRNA gene	10pM/ μ L	1.00
7	Reverse primer of <i>16S</i> rRNA gene	10pM/ μ L	1.00
8	Forward primer of <i>cadF</i> gene	20pM/ μ L	1.00
9	Reverse primer of <i>cadF</i> gene	20pM/ μ L	1.00
10	Forward primer of <i>mapA</i> gene	10pM/ μ L	1.00
11	Reverse primer of <i>mapA</i> gene	10pM/ μ L	1.00
12	Forward primer of <i>ceuE</i> gene	10pM/ μ L	1.00
13	Reverse primer of <i>ceuE</i> gene	10pM/ μ L	1.00
14	Nuclease free water		8.75
Total			30.00

influence on bacterial occurrence.

Statistical analysis

All data generated during the study were analysed using SPSS software (version 24.0). Descriptive statistics were applied to determine the frequencies, percentages, and mean values (\pm standard error) for the various parameters. Differences in the occurrence of *Campylobacter* spp. among districts and between soil types were assessed using the Chi-square test. The comparison of physicochemical parameters of soil (pH, EC and OC) based on the presence or absence of *Campylobacter* spp. was determined using the independent *t*-test. A *p*-value of less than 0.05 was considered statistically significant.

Antibiotic susceptibility testing

The antibiotic susceptibility profile of the *Campylobacter* isolates obtained from soil samples was determined using the disc diffusion method as described by Baserisalehi et al. (2007), with interpretations based on the Clinical and Laboratory Standards Institute (CLSI, 2015) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2025) guidelines.

Pure cultures of each isolate were grown on Mueller–Hinton Broth (MHB) and incubated at 42°C overnight under microaerophilic conditions. The resulting culture was adjusted to a turbidity equivalent to a 0.5 McFarland standard. A bacterial suspension was uniformly swabbed onto the agar surface (MHA with 5% defibrinated sheep blood). Antibiotic discs were placed on the plates using sterile forceps, ensuring proper spacing, and the plates were incubated under the same conditions for 24h. After incubation, the diameter of the inhibition zones was measured in millimetres, and isolates were categorised as sensitive, intermediate or resistant according to standard interpretive criteria. A total of 36 antibiotics belonging to various antimicrobial classes, including aminoglycosides, β -lactams, cephalosporins, macrolides, fluoroquinolones, tetracyclines, sulfonamides, glycopeptides and nitrofurans, were tested to provide a broad understanding of resistance trends.

Table 3. Overall occurrence of *Campylobacter* spp. in soil

Source of Soil	Thrissur			Kottayam			Thiruvananthapuram			Overall		
	Total soil samples	Positive		Total soil samples	Positive		Total soil samples	Positive		Total no.	Positive	
		No.	%		No.	%		No.	%		No.	%
Crop fields	7	0	0	13	2	15.4	4	0	0	24	2	8.3
Nestings	3	1	33.3	7	0	0	1	0	0	11	1	9.1
Zoo enclosures	10	2	20	0	0	0	9	0	0	19	2	10.5
Pet Stations	0	0	0	0	0	0	6	0	0	6	0	0
Total	20	3	15	20	2	10	20	0	0	60	5	8.3
χ^2 Value (P-value)	2.222 ^{ns} (0.329)			1.197 ^{ns} (0.274)			-			-		

** Significant at 0.01 level; ns- non-significant; * Significant at 0.05 level

The multiple antibiotic resistance (MAR) index for each isolate was calculated using the formula proposed by Krumpferman (1983): MAR index = a/b , where *a* represents the number of antibiotics to which the isolate was resistant, and *b* is the total number of antibiotics tested. Isolates showing resistance to three or more antimicrobial classes were classified as multidrug-resistant (MDR).

Results and discussion

Occurrence of *Campylobacter* spp. in soil

Out of the 60 soil samples collected from various bird-associated environments across three districts of Kerala, *Campylobacter* spp. was detected in 8.3 per cent of the samples. The district-wise occurrence was highest in Thrissur (15%), followed by Kottayam (10%), while absent in Thiruvananthapuram (Table 3). These findings indicate that *Campylobacter* can persist in certain soil microhabitats, particularly where moisture and organic matter are favourable. The present results closely align with earlier studies from Kerala, such as Athulya et al. (2021), who recorded an 8 per cent prevalence in duck-associated soils and Pravitha et al. (2022), who reported a 2.8 per cent prevalence in broiler-associated soils. The absence of positives in Thiruvananthapuram could be linked to drier soils and lower rainfall (Kerala ENVIS Centre, 2025) during the sampling period (December to March), as soil humidity and temperature significantly influence *Campylobacter* survival.

Occurrence in crop fields (Migratory bird-associated soil)

Of the 24 samples collected from crop fields and riverbanks frequented by migratory birds, *Campylobacter* spp. was detected in 8.3 per cent, restricted to Kottayam district (11.1%). This pattern reflects possible contamination by migratory waterfowl during feeding or resting. Waterfowl are known carriers of *Campylobacter*, shedding large numbers of bacteria in their droppings, which can contaminate agricultural soils and irrigation sources. Once introduced, *Campylobacter* can persist for extended periods under moist and shaded soil conditions (Jaderlund et al., 2011). Variations between districts likely

comes from ecological and behavioural differences among bird populations and from agricultural practices such as flooding or irrigation, which promote bacterial survival and dispersal.

Occurrence in nesting sites (Urban bird-associated soil)

Among the 11 soil samples obtained from urban bird nesting and roosting sites, *Campylobacter* spp. was detected in 9.1 per cent, all from Thrissur (20%). The high positivity in Thrissur may be related to dense crow and pigeon colonies nesting near residential and waste disposal areas. Urban birds such as crows have been shown to harbour *Campylobacter* spp. and their droppings can serve as a source of environmental contamination (Muralikrishna, 2018). Soils beneath nesting sites often accumulate organic debris and retain moisture, providing a microenvironment that favours bacterial persistence. Continuous faecal deposition by these birds likely enhances environmental seeding, sustaining *Campylobacter* survival cycles.

Occurrence in zoo enclosures (Captive bird-associated soil)

In the 19 soil samples collected from zoo enclosures, *Campylobacter* was detected in 10.5 per cent, confined to Thrissur (20%). Such enclosures often contain high densities of captive and free-ranging birds, leading to recurrent contamination through faecal droppings. The combination of high organic content, retained moisture and limited sunlight within these enclosures supports bacterial persistence. Casalino et al. (2022) similarly reported that faecal accumulation and inadequate enclosure sanitation facilitate the persistence of *Campylobacter* in captive wildlife settings. Hence, enclosure-level hygiene and the presence of wild bird visitors likely play a major role in maintaining *Campylobacter* in zoo-associated soils.

Occurrence in pet bird cages (Pet bird-associated soil)

All six soil samples collected from pet bird cages tested negative for *Campylobacter* spp. This may

be attributed to good management practices, including frequent cage cleaning and the use of dry substrates such as sand or litter, which hinder bacterial survival. As *Campylobacter* is highly sensitive to desiccation, its persistence is minimal under low-moisture conditions (Whiley et al., 2013). Moreover, pet birds generally have limited exposure to wild carriers and are maintained under controlled feeding and hygiene conditions, reducing the likelihood of environmental contamination.

Species identification

Molecular and biochemical characterisation confirmed *C. jejuni* as the predominant species in soil, followed by *C. coli* and other *Campylobacter* spp. (Fig. 1) This predominance of *C. jejuni* aligns with global reports that identify it as the most prevalent thermophilic *Campylobacter* species in both avian hosts and environmental samples (Chai et al., 2009). The occasional detection of *C. coli* and mixed infections suggests that environmental overlap between different avian species can facilitate co-colonisation and horizontal gene transfer within soil ecosystems.

Culture and direct broth PCR detection

Three samples that were positive by conventional culture technique were also detected by direct broth PCR. The PCR detected two more positive samples than conventional culture (in Kottayam), highlighting its superior sensitivity for detecting stressed and VBNC cells (Fig. 2). Such differences are consistent with the findings of Singh et al. (2011) and Reichelt et al. (2023), who reported that PCR-based detection is more reliable in environmental samples where bacterial cells often experience desiccation and nutrient stress that hinder culture detection. These results reaffirm the complementary value of molecular methods in ecological monitoring of *Campylobacter*.

Physicochemical characteristics of soil

The physicochemical properties of the soils varied among districts, reflecting the influence of local climatic and geological conditions. Soils from Kottayam were very strongly acidic (pH 4.5–5.0), Thrissur soils

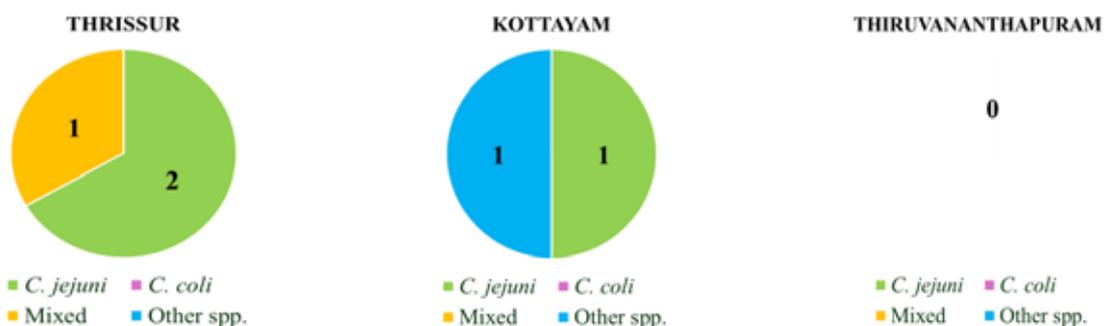


Fig. 1. Overall occurrence of *Campylobacter* spp. in bird (wild, captive and pet)-associated soils (species-wise)

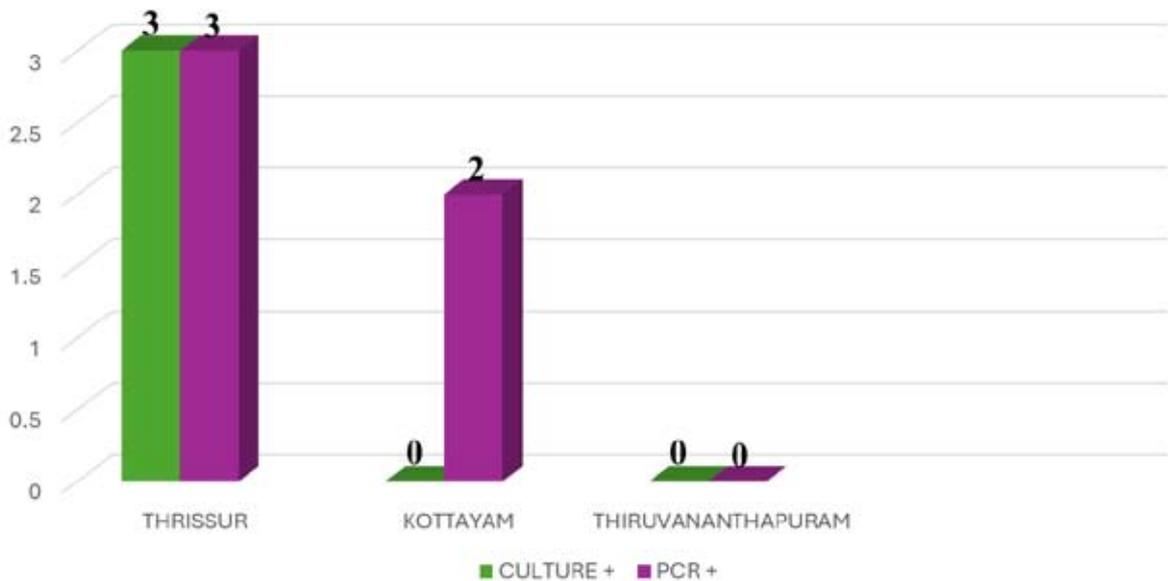


Fig. 2. Identification by culture and direct broth PCR in bird (wild, captive and pet)-associated soils

were moderately acidic (pH 5.6–6.0), and those from Thiruvananthapuram were near neutral. Organic carbon levels were high in all districts, with the highest mean value in Thiruvananthapuram (3.55%) and the lowest in Thrissur (1.87%). Electrical conductivity was highest in Thiruvananthapuram and lowest in Kottayam, indicating regional variation in soil ionic content and moisture status (Table 4).

Comparative analysis showed that *Campylobacter* positive soils tended to have slightly higher pH but significantly lower EC than soils negative for *Campylobacter*. This finding suggests that low salinity and moderate acidity may favour *Campylobacter* survival in soil microenvironments (Table 5). Similar patterns have been observed in other environmental matrices, where high EC or salinity impairs bacterial viability by inducing osmotic stress (Bronowski et al., 2014). Although the relationship between organic carbon and *Campylobacter* presence

was not statistically significant, high organic matter content likely provides protective niches for bacterial persistence by buffering against environmental fluctuations (Brandl et al., 2004; Jolly, 2022).

Antibiotic susceptibility testing of *Campylobacter* isolates

Three *Campylobacter* isolates obtained from soil from Thrissur were tested for their antibiotic susceptibility profile against 36 antibiotics representing different antimicrobial classes of clinical and veterinary relevance. All isolates were resistant to cefotaxime, clindamycin and lincomycin. Resistance was observed in 66.7 per cent of isolates to aztreonam, cefixime, ceftazidime and cephalothin, while 33.3 per cent of isolates exhibited resistance to cefepime, ceftriaxone, chloramphenicol, tetracycline and vancomycin. All isolates were sensitive to aminoglycosides (amikacin, gentamicin, kanamycin,

Table 4. Average physico-chemical characteristics of soil

District	pH	Electrical Conductivity ($\mu\text{S}/\text{cm}$)	Organic Carbon (%)
Thrissur	5.9465 \pm 0.27994	291.00 \pm 72.23	1.87 \pm 0.25
Kottayam	4.47 \pm 0.24	160.25 \pm 94.27	2.91 \pm 0.41
Thiruvananthapuram	6.59 \pm 0.27	445.35 \pm 68.18	3.55 \pm 0.58

Table 5. Association of physico-chemical parameters with the presence/absence *Campylobacter* spp.

Physico-chemical parameter	<i>Campylobacter</i> spp. absent (n=55)	<i>Campylobacter</i> spp. present (n=5)	t-value	p-value
pH	5.61 \pm 0.20	6.28 \pm 0.25	2.049 ^{ns}	0.067
Electrical Conductivity ($\mu\text{S}/\text{cm}$)	321.02 \pm 50.62	55.20 \pm 17.26	4.959**	<0.001
Organic Carbon (%)	2.83 \pm 0.28	2.19 \pm 0.74	0.809 ^{ns}	0.454

** Significant at 0.01 level; ns - non-significant; * Significant at 0.05 level

streptomycin, tobramycin), β -lactams such as amoxycyclav and ampicillin, macrolides (azithromycin and erythromycin), fluoroquinolones (ciprofloxacin, enrofloxacin, levofloxacin, norfloxacin, ofloxacin, and gatifloxacin), sulfonamides (cotrimazole), and carbapenems (imipenem, meropenem, and doripenem).

The MAR index of soil isolates ranged from 0.19 to 0.22, which indicates moderate exposure to antimicrobial agents. According to Krumperman (1983), a MAR index value greater than 0.2 reflects high-risk sources with frequent antibiotic exposure. The slightly elevated MAR indices in the present study suggest potential contamination of soil from sources such as livestock waste, poultry manure or agricultural runoff. None of the soil isolates were multidrug resistant, which may indicate a comparatively lower selective pressure in the sampled environments.

The susceptibility of the isolates to macrolides (azithromycin and erythromycin) and fluoroquinolones (ciprofloxacin, enrofloxacin, levofloxacin, norfloxacin, ofloxacin, and gatifloxacin) is a promising finding, as these antimicrobials constitute the first-line therapeutic options for human campylobacteriosis (WHO, 2020). However, studies from various parts of India have highlighted the gradual emergence of resistance to fluoroquinolones in *Campylobacter* from animal and environmental sources (Kumar et al., 2021), underscoring the importance of continuous monitoring. Despite having inherent and acquired resistance to certain antibiotics, it was observed that all the *Campylobacter* isolates tested to the various classes of antibiotics were sensitive, which may probably be due to the low number of isolates obtained in the study. Although the soil isolates in this study demonstrated relatively low overall resistance levels, soil can serve as a long-term reservoir for bacterial populations exposed to residual antibiotics or faecal contaminants.

Conclusion

The present study highlights the occurrence and antimicrobial resistance profile of *Campylobacter* spp. in soils associated with wild, captive and pet bird habitats in Kerala. The detection of *Campylobacter* in 8.3 per cent of soil samples confirms its potential role as an environmental reservoir, particularly in bird-frequented sites such as crop fields, nesting areas and zoo enclosures. *Campylobacter jejuni* emerged as the predominant species, while molecular detection proved more sensitive than culture technique, emphasising the value of PCR-based surveillance. Physicochemical findings suggest that moderately acidic soils with low electrical conductivity favour *Campylobacter* survival. Antibiotic susceptibility testing revealed that all the isolates remained sensitive to macrolides, fluoroquinolones, aminoglycosides and carbapenems. Incorporating soil surveillance into ongoing zoonotic disease and antimicrobial resistance monitoring

programmes is crucial for early detection, risk assessment and mitigation under the One Health framework.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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