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Original Article

Characterization of thermophilic *Campylobacter* species from diarrhoeic dogs referred to veterinary hospitals in Ilorin, Kwara State, Nigeria

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Abstract

Background: *Campylobacter* is a leading cause of bacterial gastroenteritis globally, and pets are important reservoirs for human infections. **Aims:** This study aimed at characterizing thermophilic *Campylobacter* from diarrhoeic dogs at major veterinary hospitals in Ilorin, Nigeria. **Methods:** In a cross-sectional approach, 120 fecal samples were collected from diarrheic dogs presented to selected Veterinary Hospitals between March and December 2022. *Campylobacter* was isolated and identified using standard bacteriological methods. Subsets of the isolates were characterized targeting *16S rRNA*, and all isolates were subjected to antimicrobial susceptibility testing. **Results:** 13.0 (10.8%) of the samples were positive for *Campylobacter*. The isolation rate of *Campylobacter* from studs (2.5%) was lower than that of the bitches (8.3%) albeit the difference was not statistically significant (P>0.05). Conversely, the rate during raining season (9.2) was significantly higher than that of dry season (1.7%) (P<0.05). The isolates showed evolutionary relationships with *Campylobacter coli* (n=2) and *Campylobacter jejuni* (n=1). Varying resistance profiles were exhibited by the isolates, with 92.3% showing resistance to ampicillin and nalidixic acid, while 84.6% resisted tetracycline. The lowest rate (7.7%) of resistance was observed to chloramphenicol, and 46.2% of the isolates displayed MDR phenotypes. **Conclusion:** This study reports *Campylobacter coli* and *Campylobacter jejuni* from dogs for the first time in Ilorin, Kwara State, and highlights the antimicrobial resistance patterns of the isolates. The presence of antimicrobial-resistant *Campylobacter* species in dogs calls for continuous surveillance, given the zoonotic potential of this organism.

Key words: *Campylobacter*, Dogs, Ilorin, Kwara, Multidrug Resistance

Introduction

Campylobacteriosis caused by *Campylobacter* species is the major cause of bacterial gastroenteritis globally (Jribi *et al*., 2017). Despite increasing rates of gastroenteritis in Nigeria, campylobacteriosis is not well documented in the region due to the inadequacy of laboratory facilities for the detection of *Campylobacter* species (Waje *et al*., 2024). The genus *Campylobacter* currently encompasses over 30 different species and subspecies (Acke, 2018). The major species commonly encountered in human and animal gastroenteritis are *Campylobacter jejuni* and *Campylobacter coli* (Goni *et al*., 2017). Other species, such as *Campylobacter upsalensis*, *Campylobacter lari*, *Campylobacter curvus*, *Campylobacter showae*, and so on, have also been incriminated in bacterial gastroenteritis at low incidence (Goni *et al*., 2017). The major source of human campylobacteriosis is consumption of contaminated poultry products (Kaakoush *et al*., 2015). Contact with pets has been identified as another major source of human campylobacteriosis, especially in Europe and America (Ghunaim *et al*., 2015). About 25% of *Campylobacter* infections in humans are associated with contact with pets (including cats and dogs) in several European countries (Amandine *et al*., 2020). Some studies indicated that contact with diarrhoeic pets predisposes to *Campylobacter* infection more than contact with healthy ones (Campagnolo *et al*., 2018), while others reported increased campylobacteriosis with the presence of puppies in the households (Carbonero *et al*., 2012). However, in developing countries, like Nigeria, the report of the association between pets and *Campylobacter* infection is scanty (Salihu *et al*., 2010).

Salihu *et al*. (2010) and Karshima and Bobbo (2016) reported *Campylobacter* species from pets in the North Western and North Central Nigeria, respectively, but the data were restricted to phenotypic characterization. To the best of our knowledge, there is no published report on *Campylobacter* species from pets in Kwara State. The major species of *Campylobacter* associated with dogs include *Campylobacter upsaliensis*, *Campylobacter jejuni*, *Campylobacter helveticus*, and *Campylobacter coli* (Acke, 2018).

The presence of *Campylobacter* species in the dogs' population poses health threats to the animals themselves in addition to their public health significance (Acke, 2018; Ahmed *et al*., 2025), hence, the need to investigate the *Campylobacter* carriage by pets requires urgent attention. Campylobacteriosis is said to influence male animals more than female ones. The reason for this phenomenon is unclear, but it may be attributed to physiological differences between the sexes (Mahmoud *et al*., 2022). The infective dose of *Campylobacter* species is estimated at 500-800 CFU, and fecal samples from dogs have been reported to contain up to 10^6 CFU/g organisms; hence, dogs play crucial roles in environmental contamination or direct human infection during contact (Facciolà *et al*., 2017). The high concentration of *Campylobacter* species in dogs' feces could probably be the reason for the quick spread of organisms among dogs kept together in close contact (Acke, 2018). The proper mechanism for transmission of *Campylobacter* from pets to humans has not been fully described. However, it was postulated that contact with pets could transfer pathogens from their contaminated fur to human hands, leading to infections (Ahmed *et al*., 2025). Hence, the need to understand the status of *Campylobacter* carriage by diarrhoeic dogs visiting veterinary hospitals is crucial, especially in developing countries like Nigeria.

Infections with *Campylobacter* species are usually characterized by a wide variety of symptoms, some of which include moderate to severe inflammatory diarrhea, abdominal discomfort, and fever. The symptoms are usually self-limiting, but in severe cases, erythromycin (macrolides), ciprofloxacin (fluoroquinolone), and tetracycline are the drugs of choice for the treatment of the infections. However, the emergence of multidrug-resistant *Campylobacter* strains has limited the therapeutic approach (Ahmed *et al*., 2025). Increased resistance of *Campylobacter jejuni* to fluoroquinolones has been reported (Ghunaim *et al*., 2015). Several studies have equally reported multidrug-resistant *Campylobacter* species from pets (Ahmed *et al*., 2025), and the resistant species can be transferred to humans directly via consumption of contaminated food or indirectly through contact with animals, including pets (Goni *et al*., 2017; Acke, 2018). Data on the resistance profiles of *Campylobacter* from pets in Nigeria is an enigma, hence, the need for this study.

Campylobacter can be detected using either culture-dependent or culture-independent methods (Fitzgerald, 2015; Kashoma *et al*., 2016). The culture-dependent method is limited by its low sensitivity, cumbersome, and time-consuming nature (Kaakoush *et al*., 2015). Non-culture-dependent methods are more sensitive and determine the genetic profile of an unknown organism from DNA/RNA extracted directly from a clinical sample using sequencing or genus-species/specific-specific polymerase chain reaction (PCR) amplification of a target gene, such as the *16S RNA* gene (Fitzgerald, 2015; Kaakoush *et al*., 2015; Ahmed *et al*., 2025). As there is no data on the status of thermophilic *Campylobacter* species from dogs presented to major hospitals in Kwara State, this study was therefore aimed at characterizing thermophilic *Campylobacter* from diarrheic dogs presented to major Veterinary Hospitals in Ilorin, Kwara State, Nigeria.

Materials and Methods

Geographical location of the study

The study was carried out in Ilorin, Kwara State, Nigeria. Ilorin is the capital of Kwara State, North Central Nigeria. Ilorin is located on latitude 8° 32´N and longitude 4° 35´ East. It has an area of about 468 km^2 and it is situated in the transitional zone between the forest and the Guinea savannah regions of Nigeria (Olabode and Oluwafemi, 2016). Kwara State is made up of 16 Local Government Areas with an estimated population of about 2,588,108 (NPC & ICF International, 2014). There are four major veterinary hospitals in Ilorin, namely: the University of Ilorin Veterinary Teaching Hospital (UVTH), the Kwara State Veterinary Hospital (KWVH), the Nigeria Police Veterinary Hospital (NPVH), and the Nigeria Prison Service Veterinary Hospital (NPSVH). Many private veterinary hospitals are spread across the length and breadth of the city (personal field experience). For convenience, three Veterinary Hospitals were selected for sampling, and these include UVTH, KWVH, and a private Veterinary Clinic (Animal's Hope Veterinary Clinics, AHVC). Kwara State was chosen for the study because of its central location and serves as the border State between the southern and northern parts of Nigeria.

Ethical approval

Ethical approval was sought from the Faculty of Veterinary Medicine Ethical Review Committee with approval number FVM/UERC/2021/15/32TA004, and each client completed a client consent form to show the owner's consent before the sample was collected from any pet.

Study design and sample size determination

The study used a prospective cross-sectional design to detect *Campylobacter* from diarrhoeic dogs presented to selected veterinary hospitals in Ilorin metropolis between March and December 2022. Sample size was determined using the Thrusfield formula:

$$n= Z^2 \times P_{exp} (1-P_{exp})/d^2$$

Where,
n: No of samples
Z^2= 1.96 for 95% confidence interval
d: The desired absolute precision
P_{exp} = Expected prevalence (8.1%, Salihu *et al*., 2010) at a confidence interval of 95% and an absolute precision of 5%

Therefore, n=113 but was rounded up to 120 to increase precision. Samples were collected from diarrhoeic dogs presented to the selected hospitals during the study period.

Sample collection

A total of 120 samples were collected from March to December 2022 as follows UVTH (n=72), KWVH (n=31), and AHVC (n=17). Among these, 80 samples (UVTH, n=50, KWVH, n=21, AHVC, n=9) were collected in the raining season, while 40 (UVTH, n=22, KWVH, n=10 AHVC, n=8) were collected in the dry season. Of the dogs sampled, 90 were females (UVTH, n=62, KWVH, n=17, AHVC, n=11) while 30 (UVTH, n=10, KWVH, n=14, AHVC, n=6) were males. The total number of samples from each Veterinary clinic was the number of diarrhoeic dogs presented within the study period. The samples from each of the Veterinary hospitals were collected weekly as cases were presented to the small animal clinics of the hospitals. Only diarrhoeic dogs were included in the study, while other dogs presented for routine vaccination or health check were excluded from the study. Faecal samples were collected per rectum using a sterile swab stick (Puritan®) or from freshly voided feces on the clinic floor. Samples were collected in sterile sample bottles containing charcoal cefoperazine deoxycholate broth (Hi-Media, Mumbai, India), as previously described (Bojanic *et al*., 2016). Samples were placed on ice and immediately transported to the Veterinary Microbiology laboratory at the University of Ilorin for analyses. The information (sex and age) of the animals sampled, as well as the period of sampling were recorded.

Isolation and identification of *Campylobacter* from the fecal sample

Isolation of *Campylobacter* from fecal samples was done according to the previously reported protocol (Mahmoud *et al*., 2022). Briefly, approximately 0.5 g of fecal sample was suspended in 4.5 ml of modified *Campylobacter* broth (Oxoid, Hampshire, UK), and incubated at 42ºC for 48 h under microaerophilic conditions provided by CampyGen (Oxoid, Hampshire, UK). After incubation, 1 ml from each inoculum was transferred into 9 ml thioglycolate broth supplemented with Preston *Campylobacter* selective supplement (SR0204E; Oxiod, Hampshire, UK) which served as the enrichment medium, and incubated at 42ºC for 48 h under microaerophilic condition provided by CampyGen (Oxoid, Hampshire, UK), after which, a loopful of the enriched culture was streaked onto modified charcoal cefoperazine deoxycholate agar (mCCDA) plate (Oxoid, Hampshire, UK) supplemented with Preston *Campylobacter* selective supplement (SR0204E; Oxoid, Hampshire, UK) and the plates were then incubated for 48 h at 42°C under microaerobic conditions (CampyGen™ Oxoid, Hampshire, UK). Distinct colonies suspected to be *Campylobacter* were selected from each mCCDA plate and further purified using Mueller-Hinton agar (Oxiod, Hampshire, UK) supplemented with Preston *Campylobacter* selective supplement (SR0204E, Oxoid, Hampshire, UK). Presumptive positive isolates on modified charcoal cefoperazine deoxycholate agar showed characteristic creamy-grey, moist, flat/spreading swarming colonies.

Suspected isolates were presumptively identified using biochemical assays, including Gram reactions, catalase, oxidase, urease, sugar fermentation, and Hippurate hydrolysis (Mahmoud *et al*., 2022). Pure isolates were stored in Mueller-Hinton broth containing 20% glycerol at -20°C until needed.

Molecular analysis of selected isolates

Molecular assays are the gold standard for detecting microorganisms, but they are not readily available in low-and middle-income countries because of their cost. Due to financial constraints, three (One presumptive *Campylobacter jejuni* and two from other species) of the isolates were randomly selected for molecular analysis. The isolates were sub-cultured from stock on mCCDA (Oxoid Hampshire, UK) and incubated under microaerophilic conditions using Campygen® (Oxoid, Hampshire, UK) at 42°C for 48 h. A discrete colony from the resulting *Campylobacter* growth was then purified on 5% sheep blood Mueller Hinton agar plates, which were incubated under microaerobic conditions using campygen® (Oxoid, Hampshire, UK) at 42°C for 24 h. The purified growth was then scooped into 1 ml charcoal broth (Oxoid Hampshire, UK) containing 20% glycerol and then frozen at -20°C for two days before being shipped to the Bioscience laboratory at the International Institute for Tropical Agriculture, Ibadan, still frozen using dry ice (BOC gas, Lagos).

DNA extraction

DNA extraction was carried out following the manufacturer's instructions using ZR fungal/bacterial DNA miniPrepTM as previously described (Akeem *et al*., 2017).

PCR and sequencing of the isolates

The PCR assay was done according to the standard protocol as described by Wagner *et al*. (2008) using the following set of primers: 16SF: (5´-TAT GGA GAG TTT GAT CCT GG-3´), 16SR: (5´-AGC GTT ACT CGG AAT CAC TG-3´) as previously reported (Paul and Trevor, 1989). The primers were selected because they target the universal *16S rDNA* gene for the identification of *Campylobacter*. The primers were synthesized at Inqaba Biotech (South Africa). The amplicon from the reaction was loaded on 1.5% agarose gel, containing 0.5 µL/ml of ethidium bromide (Pharmacia, Sweden) at 100 V for 1 h and visualized under UV light in a Gel Doc 2000 documentation system (Pharmacia, Sweden).

The amplified products were purified, and a partial genome sequence was conducted using a genetic analyzer 3730xl sequencer (ThermoScientific, UK). The consensus sequences were generated from the sequences obtained using BioEdit 7.7.1. and the consensus sequences were compared with sequences in the Gene Bank (www.blast.ncbi.nlm.nih.gov/Blast.cgi). The homologous sequences retrieved and used to construct the phylogenetic tree using MEGA version X, based on Maximum Likelihood method and Tamura-Nei model. The sequences of the characterized isolates were deposited in NCBI Gene Bank and accession numbers have been assigned (https://submit.ncbi.nlm.nih.gov/subs/?search=SUB13974064).

Antimicrobial susceptibility testing

Antibiotic sensitivity testing was performed on all presumptive *Campylobacter* isolates using the Kirby-Bauer disc diffusion assay. The following antibiotics were present on the antibiotics disc panels (Oxoid, Hampshire, UK) used; ampicillin 10 µg (AMP 10), ceftazidime 30 µg (CAZ 30), ciprofloxacin 5 µg (CIP 5), ceftriaxone 30 µg (CTR 30), chloramphenicol 30 µg (C 30), cefoxitin 30 µg (CX 30), gentamicin 10 µg (GEN 30), nalidixic acid 10 µg (NAL 10), and tetracycline 10 µg (TET 10). The antimicrobials were selected because they are the drugs commonly used in the management of systemic bacterial infection in Nigeria (Usman, 2020). Stock isolates were sub-cultured on freshly prepared 5% sheep blood agar (Oxoid, Hampshire, UK), from which one to two discrete colonies were inoculated into test tubes containing 5 ml of sterile distilled water. After standing for about 2 min, the turbidity was adjusted to 0.5% MacFarland standard.

The inoculum was poured onto the Mueller-Hinton agar plate supplemented with 5% sheep RBC and evenly spread until the entire surface was covered. The excess inoculum was then discarded. For 3-5 min, the plates were partially left open to dry. Antibiotic sensitivity discs were placed on each plate and gently pressed using a disc dispenser (Oxoid Hampshire, UK). The plates were then incubated at 42°C for 18 h under microaerophilic conditions (Campygen®). The zones of inhibition of each antibiotic were measured and recorded according to the Clinical and Laboratory Standards Institute (CLSI) standards. The zones of inhibition were interpreted as sensitive, intermediate, or resistant according to CLSI guidelines (CLSI, 2020). *Escherichia coli* CCM 3954 was used as the control strain.

Data analyses

The data generated were computed into a Microsoft Excel 2016 database. The rate of *Campylobacter* isolates from overall samples, as well as the frequency of *Campylobacter* from each sample source, was determined. The data were statistically analyzed using Open-Source Epidemiologic Statistics (OpenEpi), version 3.03a (http://www.openepi.com/Menu/OE_Menu.htm, accessed on 19/05/2021). Chi-square was used to determine the level of significance in the rates of isolation between the sex of animals and periods of sampling ($P<0.05$ was considered significant).

Results

Out of the 120 samples collected from diarrheic dogs in Ilorin, Kwara State, 13 (10.8%, 95% CI) were presumptively positive for *Campylobacter* species. The isolation rate of *Campylobacter* from the studs (2.5%; 95% CI, n=3) was lower than that of the bitches (8.3%; 95% CI, n=10), even though the difference was not statistically significant ($P>0.05$) (Table 1). The rate of isolation of *Campylobacter* during the rainy season (9.2%; 95% CI) was significantly higher than that of the dry season (1.7%; 95% CI) ($P<0.05$) (Table 2).

All the isolates showed the typical characteristics of *Campylobacter* in different biochemical assays, including rapid motility, Gram-negative curved rods, catalase and oxidase positive, and none of the sugars tested was fermented. Only 2 (15.4%) isolates were presumptively identified as *Campylobacter jejuni* based on Hippurate hydrolysis (Table 3).

Two of the characterized *Campylobacter* isolates clustered with *Campylobacter coli* while the third one clustered with *Campylobacter jejuni* reference strains from the Gene Bank. They all clustered distinctly away from the outgroup (*Arcobacter* species) (Fig. 1).

A varying degree of resistance was observed among the isolates, with 92.3% of the isolates exhibiting resistance to ampicillin and tetracycline, while 84.6% and 76.9% of the isolates were resistant to tetracycline and ceftazidime, respectively. All the presumptive *Campylobacter jejuni* (100%) were resistant to nalidixic acid, while 90.9% of all the isolates exhibited resistance to it. The lowest rate of resistance was observed with chloramphenicol and gentamicin, with only 1 (7.7%) and

Table 1: Isolation rate and sex distribution of *Campylobacter* species from diarrhoeic dogs in Ilorin, Kwara State

Sample source	No of samples		No (%) of positive		Total (%)	P-value
	Sex		Sex			
	Male	Female	Male	Female		
UVTH	10 (8.3)	62 (51.7)	1 (33.3)	5 (50.0)	6 (46.2)	
KWVH	14 (11.7)	17 (14.2)	2 (66.7)	2 (20.0)	4 (30.8)	
AHVC	6 (5.0)	11 (9.1)	0 (0)	3 (30.0)	3 (23.0)	
Total	30 (25)	90 (75)	3 (2.5)	10 (8.3)	13 (10.8)	
Ground total	120 (100)		13 (10.8)			0.2629*

UVTH: University of Ilorin Veterinary Teaching Hospital, KWVH: Kwara State Veterinary Hospital, and AHVC: Animal Hope Veterinary Clinic. * $P>0.05$

Table 2: Seasonal distribution of *Campylobacter* species from diarrhoeic dogs in Ilorin, Kwara State

Sample source	Season				Total	P-value
	Rainy season	Dry season	Rainy season	Dry season		
	No of samples collected (%)		No of positive (%)			
UVTH	50 (41.7)	22 (18.3)	6 (5.0)	0 (0.0)	6 (5.0)	
KWVH	21 (17.8)	10 (8.3)	4 (3.4)	0 (0.0)	4 (3.4)	
AHVH	9 (7.5)	8 (6.7)	1 (0.8)	2 (1.7)	3 (2.5)	
Subtotal	80 (66.7)	40 (33.3)	11 (9.2)	2 (1.7)	13 (10.8)	
Total	120 (100)		13 (10.8)			0.01946*

No: Number, UVTH: University of Ilorin Veterinary Teaching Hospital, KWVH: Kwara State Veterinary Hospital, AHVC: Animal's hope Veterinary Clinic, and * P<0.05

Table 3: Biochemical characteristics of *Campylobacter* species from diarrhoeic dogs in Ilorin, Kwara

Sample source	No of positive	GRA	Biochemical tests carried out on isolates								Detection (%)
			Motility	OXI	GLU	LAC	CAT	SUC	HIP		
									+*	-	
UVTH	6 (46.2)	G-ve˜	+	+	-	-	+	-	2 (15.4)	4 (30.8)	0.06
KWVH	4 (30.8)	G-ve˜	+	+	-	-	+	-	0(0.0)	4 (30.8)	0.04
AHVC	3 (23.0)	G-ve˜	+	+	-	-	+	-	0(0.0)	3 (23.1)	0.03
Total	13 (100.0)								2 (15.4)	11 (84.6)	0.13

GRA: Gram reactions, G-ve˜: Gram negative curved rods, CAT: Catalase, OXD: Oxidase, GLU: Glucose, LAC: Lactose, -: Negative, +: Positive HIP: Hippurate hydrolysis, SUC: Sucrose, and **Campylobacter jejuni*

Table 4: Frequency of resistance of *Campylobacter* species isolated from diarrhoeic dogs in Ilorin, Kwara State

Antimicrobials	No. of resistant isolates (%) (n=13)	*Campylobacter jejuni* (n=2)	Other *Campylobacter* species (n=11)
AMP 10	12 (92.3)	2 (100)	10(90.9)
CAZ 30	10 (76.9)	1 (50)	9 (81.8)
CRO 30	4 (30.8)	0 (0)	4 (36.4)
TE 10	11 (84.6)	2 (100)	9 (81.8)
NA 10	12 (92.3)	2 (100)	10 (90.9)
C 30	1 (7.7)	0 (0)	1 (9.1)
CX 30	5 (38.5)	1 (100)	4 (36.4)
CN 10	2 (15.4)	0 (0)	2 (18.2)
CIP 5	3 (23.1)	1 (50)	2 (18.2)

AMP: Ampicillin, CAZ: Ceftazidime, CRO: Ceftriaxone, TE: Tetracycline, NA: Nalidixic acid, C: Chloramphenicol, CX: Cefoxitin, CN: Gentamicin, and CIP: Ciprofloxacin

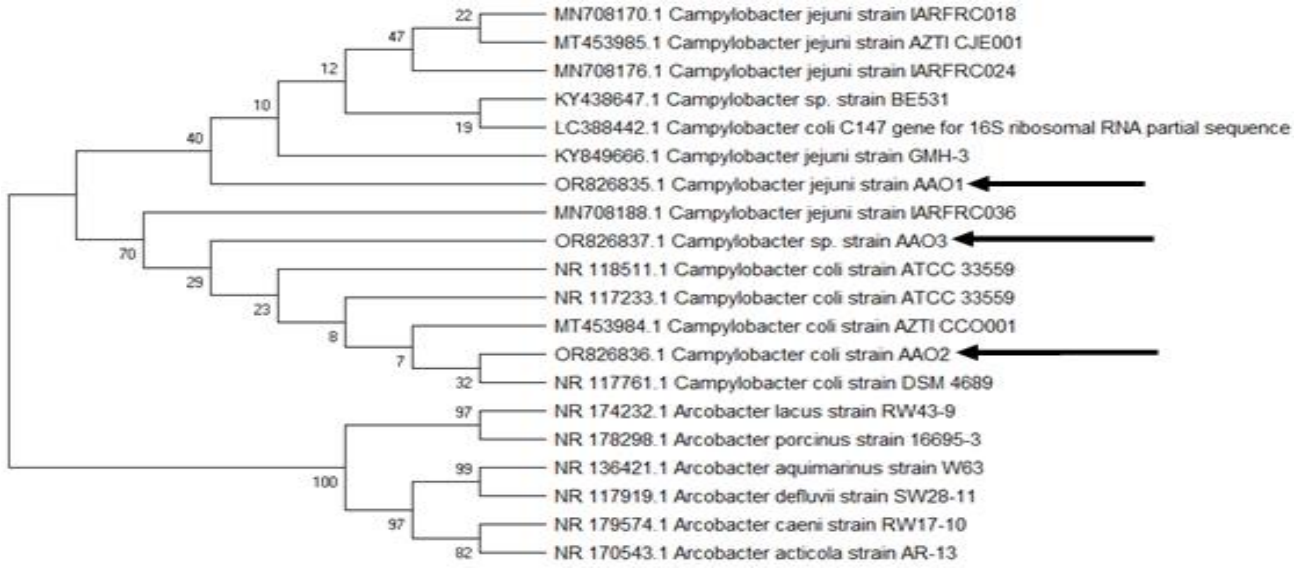

Fig. 1: Phylogenetic tree generated from *Campylobacter* species and *Arcobacter* species (outgroup) typed strains from NCBI (www.blast.ncbi.nlm.nih.gov/Blast.cgi) and consensus sequences from diarrheic dogs' isolates (two of the isolates, OR826836.1 and OR826837.1 associated with *C. coli* while OR826835.1 associated with *C. jejuni* at a sequence similarity threshold of 98.2% and based on 1000 bootstrap values. They distinctly dissociated with the outgroup (*Arcobacter* species)

Table 5: Resistance profiles of *Campylobacter* species isolated diarrhoeic dogs in Ilorin, Kwara State

S/N	Resistance profiles (No. of isolates)	No of antimicrobials resisted	Classes of antimicrobials resisted (No.)	MDR status
1	Amp-Caz (2)	2	β-Lactams (1)	Absent
2	Amp-Caz-Te (4)	3	β-Lactams-Tetracycline (2)	Absent
3	Amp-Caz-Te-Na-Cip (3)	5	β-Lactams-Tetracycline-Quinolones (3)	Present
4	Amp-Caz-Te-Na-Cn-Cip (1)	6	β-Lactams-Tetracycline-Quinolones-Aminoglycosides (4)	Present
5	Amp-Caz-Cro-Te-Na-Cn-Cip (1)	7	β-Lactams-Tetracycline-Quinolones-Aminoglycosides (4)	Present
6	Amp-Caz-Cro-Te-Na-C-Cx-Cn-Cip (1)	9	β-Lactams-Tetracycline-Quinolones-Phenicols-Aminoglycosides (5)	Present

No of isolates with MDR Phenotypes = 6, and MDR = 46.2%

2 (15.4%) showing resistance, respectively, to the antimicrobials (Table 4).

Generally, the *Campylobacter* isolates tested exhibited six different resistance patterns. The predominant phenotypes were Amp-Caz-Te (30.8%) and Amp-Caz-Te-Na-Cip (23.1%), while 1 (7.7%) isolate each was pan-resistant (resistant to all antimicrobials) and pan-susceptible (susceptible to all antimicrobials), respectively. 6 (46.2%) isolates showed multidrug-resistant phenotypes (Table 5).

Discussion

Campylobacter species is one of the most common causes of bacterial gastroenteritis globally, and it is frequently encountered in food animals, the environment, and pets (Kaakoush *et al*., 2015; Amandine *et al*., 2020; Liu *et al*., 2022). This study characterized *Campylobacter* species from diarrhoeic dogs in Kwara State. The rate of isolation (10.8%) of *Campylobacter* in the current study corroborates with the report of Salihu *et al*. (2010) but low compared to other studies conducted in different parts of the world where the rate of isolation ranged from 26%-38% in Nigeria, India, New Zealand, Iran, Texas, and Italy (Giacomelli *et al*., 2015; Bojanic *et al*., 2016; Karshima and Bobbo, 2016; Leahy *et al*., 2017; Ahmed *et al*., 2018; Torkan *et al*., 2018; Peter *et al*., 2020). but higher compared to 4.81%, which was recorded in Poland (Andrzejewska *et al*., 2013). These differences may be due to differences in the geographical location of the studies, immune status of the studied population, age of the sampled animal, method of detection, and differences in sample size, as high sample sizes have been attributed to high rates of isolation (Leahy *et al*., 2017). Breed differences are also common dictators of animals being carriers of *Campylobacter* (Bojanic *et al*., 2016). The current study also reveals that the isolation rate from studs (2.5%) was lower than that of the bitches (8.3%), even though the difference was not statistically significant. This is consistent with reports from previous studies (Salihu *et al*., 2010; Amandine *et al*., 2020; Peter *et al*., 2020) which reported that there is no sex predisposition in campylobacteriosis, however, Karshima and Bobbo (2016) reported a significant difference ($P<0.05$) between females (30.4%) and males (15%) dogs presented to veterinary hospitals in Jos, Plateau State. By contrast, the isolation rate during the rainy season (9.2%) was significantly higher than that of the dry season (1.7%) in the current study. Varying results have been reported about *Campylobacter* recovery with the seasons of sampling. While some studies reported that season does not affect the recovery rate of *Campylobacter* (Bojanic *et al*., 2016; Amandine *et al*., 2020). Others reported that the rate of recovery was higher during the rainy season when the humidity is higher than during the dry season when the temperature is high (Acke, 2018; Torkan *et al*., 2018). It is, therefore, reported that a large-scale epidemiological study is required to establish the true correlation between the rate of recovery of *Campylobacter* and the season of study (Torkan *et al*., 2018). The *Campylobacter* species obtained from diarrhoeic dogs in the current study showed an evolutionary relationship with *Campylobacter coli* and *C. jejuni* from the NCBI gene bank, signifying that they are genetically related to these strains. *Campylobacter jejuni* and *C. coli* have equally been reported from both diarrhoeic and healthy dogs in Nigeria and many other parts of the world (Salihu *et al*., 2010; Bojanic *et al*., 2016; Karshima and Bobbo, 2016; Torkan *et al*., 2018; Amandine *et al*., 2020). *C. upsalensis* which has been reported to be the commonest species of *Campylobacter* from dogs in the past centuries (Everest, 1992) was not detected in our current studies even though, *C. jejuni* and *C. coli* are, of recent, the commonly reported species from pets (Andrzejewska *et al*., 2013; Bojanic *et al*., 2016; Acke, 2018; Torkan *et al*., 2018; Amandine *et al*., 2020) The detection of *Campylobacter* species from diarrhoeic dogs may not be the major cause of diarrhea observed in the dogs even though, research has shown that experimental infection of dogs with *C. jejuni* caused disruption of gut flora leading to enteritis, colic, and fever (Bojanic *et al*., 2016; Acke, 2018). The occurrence of *Campylobacter* species in dogs has zoonotic potential and may serve as a source of environmental contamination and human infections (Acke, 2018; Torkan *et al*., 2018). The isolates detected in this current study share genetic relatedness with species that are pathogenic in humans. Close to 80-90% of human campylobacteriosis is due to *C. jejuni* and *C. coli* (Platts-Mills *et al*., 2014; Skarp *et al*., 2015). The infection of dogs with *Campylobacter* species may result from poor hygienic practices such as consumption of raw food of animal origin, and ingestion of unclean and infected foods, as previously reported (Karshima and Bobbo, 2016). The isolates were distinctly dissociated from *Arcobacter* species in the phylogenetic tree, showing they are not evolutionarily related.

The isolates showed varying degrees of resistance to commonly used antimicrobials, with a high proportion of them exhibiting resistance to ampicillin, nalidixic acid, ceftazidime, and tetracycline. Tetracycline-resistant strains of *Campylobacter* have been reported in dogs

(Rahimi *et al*., 2017; Torkan *et al*., 2018). Resistance to ampicillin, ceftazidime, and nalidixic acid by *Campylobacter* species also agreed with the results of previous studies (Torkan *et al*., 2018; Mahmoud *et al*., 2022). The recovery of resistant strains of *Campylobacter* from dogs is of public health significance as pets are potential sources of antimicrobial-resistant pathogens to humans (Acke, 2018). The increased occurrence of antimicrobial-resistant pathogens in pets usually arises from indiscriminate use and abuse of a varieties of antimicrobials in these animals for therapeutic or prophylactic purposes (Torkan *et al*., 2018). Even though it is no longer widely used, resistance to nalidixic acid is one of the phenotypic characteristics traditionally used to identify *Campylobacter* species (Mahmoud *et al*., 2022). A low level of resistance was observed to chloramphenicol and gentamicin in this study. This is in agreement with previous studies (Rahimi *et al*., 2017; Torkan *et al*., 2018). The low level of resistance to chloramphenicol may be due to the fact that the use of this drug to treat animal diseases is banned in Nigeria.

The presence of a high frequency of resistance phenotypes among the *Campylobacter* isolates from diarrheic dogs in study areas, coupled with the occurrence of multidrug resistance, showed potential failure of chemotherapy, which may not be unconnected with abuse and indiscriminate use of antibiotics in the area. Other studies have equally documented multidrug-resistant *Campylobacter* from pets in different regions of the world (Bojanic *et al*., 2016; Acke, 2018; Torkan *et al*., 2018; Amandine *et al*., 2020).

The limitations of this study include the small sample size and limited study areas, which may restrict the generalisability of the findings. Another limitation is the inability to perform molecular characterisation of all isolates due to financial constraints. Hence, further study is recommended using a large sample size covering a wide study area involving full characterization of the isolates.

This study established the presence of *Campylobacter* in diarrheic dogs presented to major veterinary hospitals in Ilorin, Kwara State, at the rate of 10.8%. The resistance profiles and phylogenetic relationship of the isolates with the existing ones were established, and they showed relationships with *Campylobacter jejuni* and *Campylobacter coli*. The research also revealed that dogs can serve as a source of drug-resistant *Campylobacter* to the environment and human infections.

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Conflict of interest

The authors do not have any conflict of interest to declare.

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Use of generative AI and AI-assisted technologies statements

No AI technology was employed in the course of preparing this manuscript.

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