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First Molecular Characterization and Antimicrobial Resistance Profiles of *Campylobacter jejuni* Isolated from Poultry Meat in Yemen

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Abstract

Background: *Campylobacter jejuni* is a foodborne bacterial infection that is of global concern and responsible for 90% of campylobacter-associated diarrheal diseases in humans. To date, there is no data on the prevalence of *C. jejuni* in poultry meat in Yemen. This study is the first one that aimed to molecularly detect and determine the antimicrobial susceptibility pattern of *C. jejuni* isolates from poultry meat in Sana'a, the capital of Yemen. **Methods:** Three hundred and thirty samples of poultry meat (180 local and 150 imported) were collected from various fresh poultry shops and slaughterhouses in the Yemeni capital, Sana’a, during the period from 2023 to 2024. *C. jejuni* isolates were identified using phenotypic methods and confirmed using molecular techniques, including polymerase chain reaction (PCR), sequence alignment, and phylogenetic analysis. The Kirby-Bauer method was used to determine the antimicrobial susceptibility profile of the isolates. **Results:** The overall rate of *C. jejuni* was detected in 40/330 (12.12%) poultry meat samples, 38/180 (21.1%) local meat, and 2/150 (1.3%) imported meat (P = 0.003; Odds Ratio (OR) =6.57). From local meat, 18 (10%) isolates were recovered from intestinal samples, and only two were recovered from skin samples (1.3%) of the imported meat. The prevalence of *C. jejuni* peaked in the autumn (12, 3.7%; OR =1.92). All *C. jejuni* isolates were completely resistant to macrolide antibiotics and clindamycin and highly resistant to aminoglycosides. In addition, the sensitivity of *C. jejuni* isolates was reported to be 100% for ampicillin and chloramphenicol and 90.0% for ciprofloxacin. All *C. jejuni* isolates exhibited multidrug resistance, with the majority being resistant to five antibiotics (60%). **Conclusions:** These findings revealed that multi-antibiotic-resistant *C. jejuni* was recovered from poultry meat, particularly local meat. Consequently, it is imperative to establish policies that will mitigate the transmission of animal diseases, restrict the use of antibiotics in poultry farming, provide slaughterhouse employees with training on the proper handling of meat, and enforce strict standards for meat imports.

Keywords: *Campylobacter jejuni*, Multidrug resistance (MDR), 16S rRNA sequencing, Conflict setting, Poultry meat, Yemen

Introduction

Campylobacter bacteria are widely distributed in food animals, such as cattle and livestock, and pets such as dogs and cats. Humans acquire the bacteria through the consumption of unpasteurized or contaminated milk, undercooked poultry products, or contaminated drinking water. Between 50 and 70% of human infections with *Campylobacter* are acquired through the consumption of poultry [2].

There are four species of *Campylobacter* bacteria that are pathogenic to humans, including *C. jejuni*, *C. coli*, *C. lari,* and *C. fetus*. Moreover, *C. jejuni* and *C. coli* are the most common and widespread in humans and cause diarrheal diseases [3]. People with *Campylobacter* infections (Campylobacteriosis) frequently experience fever, diarrhea, and stomach pain; occasionally, they may also experience nausea and vomiting [3–4]. Campylobacteriosis is typically self-limiting, with a fatality rate of five in 10,000 cases and a hospitalization requirement of 5-10% [5]. In rare cases, *C. jejuni* infection can lead to a number of complications, including Guillain-Barré syndrome, myocarditis, and acute reactive arthritis [2].

Globally, *Campylobacter* is one of the four main causes of diarrheal diseases, at 8.4% [6], with an incidence rate of 20 cases per 100,000 people in 2020 [7]**.** Moreover, *C. jejuni* and *C. coli* account for 91% and 8% of *Campylobacter* infections, respectively [8]. Furthermore, the prevalence of *C. jejuni* infections ranges from 0.3% to 2.9% worldwide. In developed countries, the incidence rate of *C. jejuni* is estimated to be 4.4–9.3 cases per 1,000 individuals [8]. In 2022, an estimated 140,241 confirmed cases of campylobacteriosis were reported in 30 EU/EEA countries, with a case-fatality rate of 0.04% [9].

A previous study revealed a widespread distribution of virulence genes among *C. jejuni* isolates from poultry and demonstrated high genetic similarity to *C. jejuni* isolated from humans, confirming that poultry meat contaminated with this bacterium poses a risk to human health [10–13]. According to previous reports, the spread of *C. jejuni* strains isolated from the environment and animals (poultry) that are resistant to multiple antibiotics has been observed. This raises public health concerns regarding the possibility of its transmission to humans, increasing morbidity rates due to difficulty, or significantly reducing the effectiveness of available treatments [14–17].

In Yemen, which continues to face a humanitarian tragedy due to the deteriorating economic and health conditions resulting from the ongoing war and restrictions imposed for more than nine years [18–20], the application of public health standards and food safety precautions is absent in slaughterhouses and local markets. Furthermore, the issue is further exacerbated by the failure of slaughterhouse workers to adhere to safety procedures and their lack of awareness regarding the potential for meat contamination with pathogens. This exposes the population to additional health risks, due to poor healthcare provision and difficulty accessing treatment [21–22].

Despite the global importance of *C. jejuni* **[**23**]**, molecular studies on its prevalence and multidrug resistance patterns in Yemen's meat supply chains remain extremely scarce, hindering the implementation of effective food safety measures. So, there is an urgent need to address this knowledge gap and establish a database on the prevalence, molecular characteristics, and antibiotic resistance of *C. jejuni* in meat sold in Yemen. This database will inform food safety policies and help reduce the risk of foodborne diseases. To the best of our knowledge, this study is the first to be aimed at molecularly characterizing and determining the antimicrobial susceptibility profiles of *C. jejuni* isolates from poultry meat in Sana'a, the capital of Yemen. The findings of this study will help slaughterhouse workers understand how important it is to follow hygiene and safety rules, stop the spread of meat-borne diseases, make food safer, and protect public health as a top priority.

Materials and methods

Study area and period

This study was a cross-sectional study conducted in Sana'a, the capital of Yemen, during one year, from 2023 to 2024. The Capital Municipality—Sana'a—consists of ten administrative districts. The geographical representation of poultry consumption was guaranteed by the collection of meat samples from six of these districts: Sha'oub, Old Sana'a, Al-Wahda, Al-Tahrir, Maeen, and Al-Thowra districts. In addition, there are many fresh poultry shops and slaughterhouses that sell meat at retail, operating with a primitive system that lacks oversight, awareness, and qualified personnel to handle meat in appropriate ways that ensure food safety and prevent contamination with pathogens. Moreover, the majority of Yemenis prefer poultry meat because it is inexpensive compared to the double-priced beef and cattle meat. Therefore, this necessitates conducting a study on the causes of diseases transmitted through poultry, which is a pressing need for public health.

Sample Size

Given the lack of previous studies in Yemen, a previous study in Saudi Arabia, which showed a Campylobacter prevalence of 26.4% in poultry meat [24] was used. The sample size was calculated using Cochrane's formula ($n = Z^2 P(1-P)/d^2$) with a 95% confidence level and a 5% margin of error, indicating a required sample size of 298. The sample size was increased to 330 in our study (330), ensuring the strength of the study and the representativeness of the population.

Sample strategy collection

The study included 330 samples to ensure an equitable distribution and avoid spatial bias. This study employed a stratified random sampling methodology. Out of these, 180 meat samples were collected from locally sourced poultry meat, specifically from five anatomical parts: skin, muscle, heart, liver, and intestines. The remaining 150 samples (skin and muscle) were collected from imported poultry purchased from retail outlets. Samples of locally sourced poultry meat were collected in sterile plastic bags and immediately transported to the laboratory for bacteriological testing. For imported meat samples of Brazilian origin, whole chicken samples were collected frozen and transported to the laboratory in refrigerated containers.

Isolation and identification of *Campylobacter jejuni*

The isolation and identification of *Campylobacter* species from meat samples was performed based on ISO10272-1:2017 guidelines [25–26]. The frozen chicken samples were left at room temperature to thaw before the parts targeted for testing were collected. In summary, 25 grams of each meat sample (skin, muscle, heart, liver, and intestine) were homogenized in 225 mL of Bolton selective enrichment broth (TM Media Titan Biotech Ltd., India), which contained 5% defibrinated horse blood. The mixture was then incubated at 37°C for 6 hours, followed by an additional 44 hours at 41.5 °C under microaerophilic conditions in an incubator with 5% O_2, 10% CO_2, and 85% N_2. After the incubation period, a loopful of the inoculum from the enriched broth was streaked on modified charcoal cefoperazone deoxycholate agar (mCCDA) plates (Neogen Corporation, UK) and *Campylobacter* Agar Base plates (Neogen Co., UK) containing 7% defibrinated horse blood and incubated at 41.5°C for 44 h under microaerophilic conditions. Thereafter, colonies suspected to be *Campylobacter* sp., based on colony morphology were picked and re-streaked onto *Campylobacter* Agar Base plates, and the plates were incubated at 41.5°C for 44 h under microaerophilic conditions [26].

Furthermore, a series of biochemical tests were performed to confirm the *C. jejuni* isolates, including Gram staining, motility, oxidase, catalase, the hippurate test, and the indoxyl acetate test. In addition, growth was conducted aerobically at 42°C and microaerobically at 25°C in accordance with the ISO 10272-1:2017 method **[**26**]**. Additionally, the identified *C. jejuni* isolates were kept in nutrient broth with 15% glycerol at -70°C for subsequent molecular verification [27].

Molecular identification

DNA extraction

DNA was extracted from *C. jejuni* isolates grown in Luria-Bertani broth (Thermo Scientific USA) for 24 h at 42°C in a microaerophilic environment.

Furthermore, genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega Co., Madison, USA) according to the manufacturer’s recommendations.

PCR amplification of 16S rRNA gene

The PCR was employed to amplify the 16S rRNA gene and was performed using universal bacterial forward primer 8F (5′-CAG GCC TAA CAC ATG CAA GTC-3′) and reverse primer 1492R (5′-GGG CGG GGT GTACAA GGC-3′) as described earlier by El-Sayed *et al*. **[**28**]**. The PCR mixture was prepared in a volume of 50 µl and contained 22 µl of MQ, 25 µl of DreamTaq Green DNA Polymerase (Thermo Fisher Scientific Inc., US), 1 µl of each forward and reverse primer (10 µmol/L), and 1 µl of template. Furthermore, the reactions were performed using an Applied Biosystem Thermal Cycler (Thermo Fisher Scientific Inc., US) and the conventional PCR conditions employed for amplifying the 16S rRNA gene were summarized in Table 1. Additionally, the amplified PCR products were electrophoresed on a 1% agarose gel (for 45 minutes at 80 volts) with a 2.5 kbp DNA ladder. The agarose gel was stained with ethidium bromide and visualized using a UV transilluminator.

Table 1. Optimized PCR thermal cycling conditions used for the amplification of the 16S rRNA gene in *C. jejuni* isolates

Phase	Temperature	Time	Cycles
Denaturation	95 °C	4 min	1
Denaturation	94 °C	30 s	35
Annealing	52 °C	30 s	30
Extension	72 °C	1 min	-
Final Extension	72 °C	10 min	1

°C = Celsius degree, **min** = Minute, **s** = Second

Sequence alignment, phylogenetic analysis, and bioinformatics analysis

The amplified PCR product was purified and sequenced at Macrogen, Korea. Raw sequencing data were edited (contig and peak chromatogram verification) using the Finch T.V. 1.4.0 program. The 16S rRNA sequences of the strain were analysed using the BLAST (N) program of the National Center for Biotechnology Information (NCBI) (Rockville Pike, Bethesda, MD, USA). Multiple sequence alignments were performed using ClustalW 2.1. Phylogenetic trees were constructed using the neighbour-joining method in MEGA X [29].

Antimicrobial susceptibility testing

All *C. jejuni* strains that were isolated from meat samples were subjected to antimicrobial susceptibility testing using the Kirby-Bauer method for disk diffusion on Mueller-Hinton Fastidious (MH-F) agar (supplemented with 5% defibrinated horse blood), according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) **[**30**]**. A sterile saline solution was used to prepare the bacterial suspension, which was then adjusted to 0.5 McFarland standard (1.5×10^8 CFU/mL) by adding a small number of freshly cultured colonies. A sterile cotton swab moistened with the prepared suspension was used to streak the MH-F medium plate in three different directions. The plate was then left for ten minutes. Then, the commercially available antibiotic discs (TM Media Titan Biotech Ltd., India), including Azithromycin (AZM, 15 µg), Erythromycin (E, 15 µg), and Ciprofloxacin (CIP, 5 µg), are used as first-line treatment in humans. Ampicillin (AMP, 10 µg), tetracycline (TE, 30 µg), and streptomycin (S, 10 µg) are commonly used in poultry production in Yemen. Chloramphenicol (C, 30 µg), clindamycin (CD, 2 µg), and gentamicin (GEN, 10 µg) are alternative therapeutic options, as well as nalidixic acid (NA, 30 µg) to detect the onset of resistance to older quinolones. The antibiotics were aseptically placed on the agar surface of the MH-F plates and incubated at 42°C for 24 hours in a microaerophilic atmosphere (10% CO_2, 5% O_2, and 85% N_2). After incubation, the diameter of the inhibition zone was measured in millimetres using a ruler, and the results were interpreted as sensitive (S) or resistant (R) based on the EUCAST guidelines (2023) [30].

Statistics analysis

The Statistical Package for Social Sciences, SPSS (Version 26, IBM®, USA) statistical program was used to analyze the obtained data. Descriptive analysis was used, and the results obtained were presented in frequencies and percentages in tables and figures. The Chi square (χ^2), Fisher's Exact Test, and 95% Confidence Interval (95% CI) were used to compare between studied variables. The Odds Ratio (OR) was also used to assess the correlations between the sample source, the season, and the prevalence of *C. jejuni* bacteria in meat poultry. A probability value (*P*) of less than 0.05 was considered statistically significant.

Results

In this study, 330 poultry meat samples were used, including 180 local samples of Yemeni origin and 150 imported samples. These results revealed that *C. jejuni* was isolated from 40/330 (12.12%) meat samples, of which 38/180 (21.1%) were local and 2/150 (1.3%) were imported. The results showed statistically significant differences between the local and imported poultry meat samples ($P = 0.003$; OR = 6.57). Statistical analyses indicated a significant variation in contamination levels of *C. jejuni* among different organs of local poultry meat ($P < 0.005$), with the intestines showing the highest percentage of *C. jejuni* isolates (10%, 18 samples), followed by skin samples (5%, 9 samples), then 2.8% each for heart and liver samples, while the lowest percentage was recorded in muscle samples (0.6%). No statistically significant difference was observed between imported meat organs, with only two skin samples showing a positive result for *C. jejuni* (1.3%), as shown in Table 2.

Table 2. Distribution of *C. jejuni* isolates across local vs. imported poultry meat sold in Yemen

Origin of poultry meat	Sources	Total sample	Positive (n=40) No. (%)	95% CI	***P*-value**	***P*-value (OR)**
Local (n=180)	Skin	180	9 (5.0)	1.83–8.17	0.000	0.003 (6.57)
	Muscle	180	1 (0.6)	0.00–1.64		
	Heart	180	5 (2.8)	0.38–5.18		
	Liver	180	5 (2.8)	0.38–5.18		
	Intestine	180	18 (10.0)	5.61–14.39		
Imported (n=150)	Skin	150	2 (1.3)	0.00–3.17	0.999	
	Muscle	150	0 (0.0)	0.00–0.00		

95% CI: 95% Confidence Interval; **OR =** Odds Ratio; ***P* value**: Probability value <0.05 (significant)

Detection of *C. jejuni* by gel electrophoresis and PCR

Figure 1 shows the DNA extracted from *C. jejuni* isolates that were run on agarose gel electrophoresis, and the positive *C. jejuni* isolates were detected in a band length of 1,500 bps.

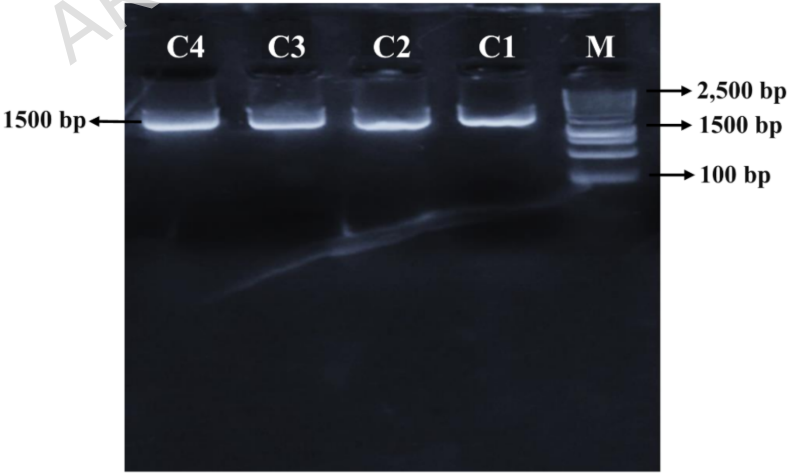

Figure 1. Agarose (1%) gel electrophoresis of DNA extracted from *C. jejuni* isolates: **Lane M:** DNA marker (100 bp – 2.5 kb); **Lanes C1–C4:** Positive *C. jejuni* isolates showing bands at the expected size of 1500 bp.

Sequence Analysis and Phylogeny of *C. jejuni* Isolates

Sequence analysis of the 16S rRNA gene revealed that the isolates taxonomically belonged to the genus Campylobacter and were closely related to *C. jejuni*. The phylogenetic tree illustrates the taxonomic placement of the Yemeni isolates within the *C. jejuni* cluster (Figure 2). Specifically, isolate No. (1) showed 98.54% sequence identity to the reference strain *C. jejuni* subsp. *jejuni* ATCC 33560 (Accession No. NR_118520.1) and was submitted to GenBank as *C. jejuni* H1 (Accession No. PV263587.1). Furthermore, isolates No. (2) and No. (4) exhibited the highest sequence similarity (97.21% and 95.62%, respectively) with *C. jejuni* subsp. *doylei* strain LMG 8843 (accession no. NR_043599.1). The isolates No. (2) and No. (4) were deposited in GenBank under the accession numbers PV263606.1 and PX392582.1, respectively.

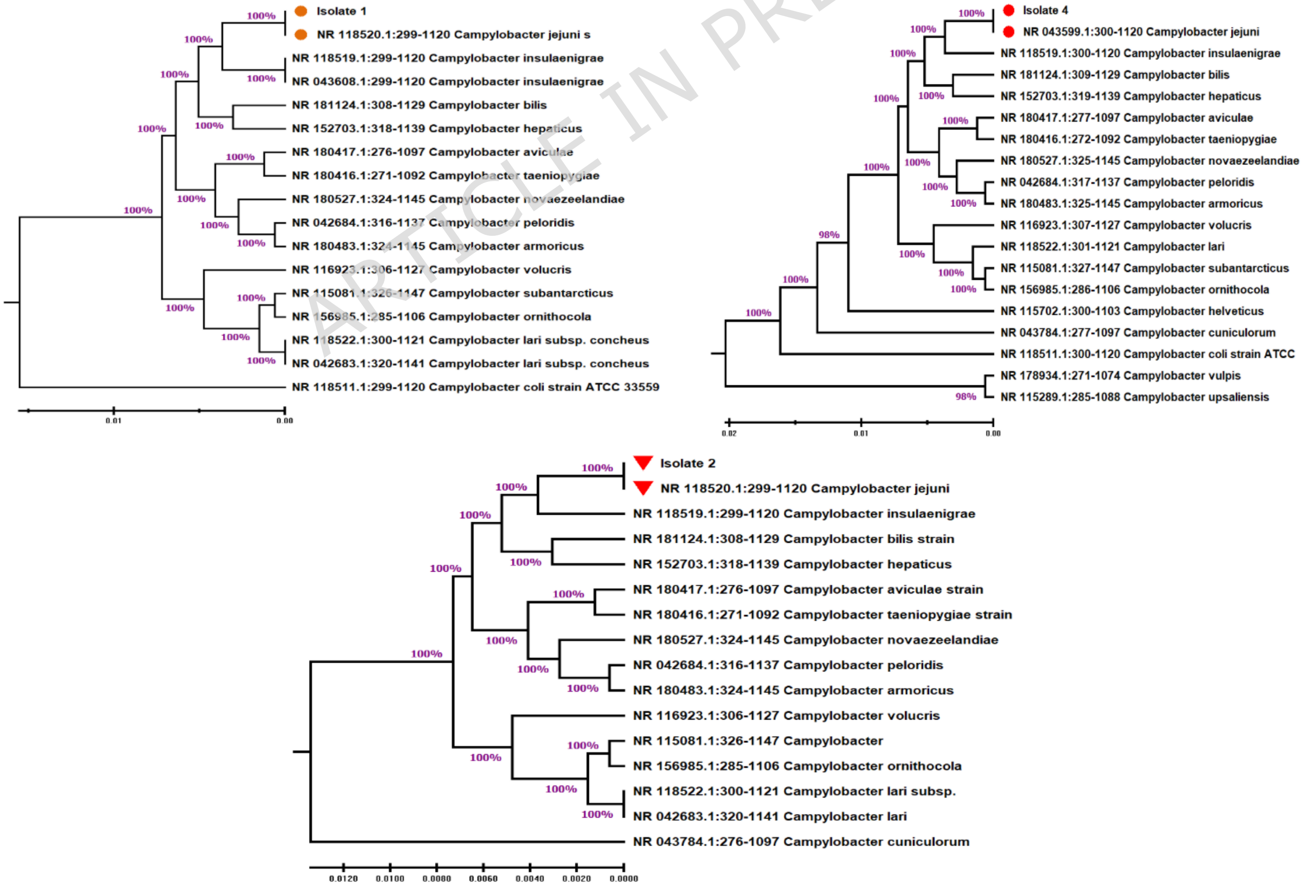

Figure 2. Phylogenetic relationships of *C. jejuni* isolates 1, 2, and 4 based on partial 16S rRNA gene sequences, showing their taxonomic placement among reference *Campylobacter* species from the GenBank database

Frequency of *C. jejuni* by seasons

Although the chi-square test indicated a significant variability between the number and prevalence of *C. jejuni* bacteria and seasonal changes ($P = 0.001$; χ^2 = 43.54), the binary odds ratios (OR) did not show any statistically significant differences between seasons ($P < 0.05$) for each season individually. The highest number of *C. jejuni* isolates was recorded in autumn (12, 3.7%; OR =1.92), followed by summer (13, 3.7%; OR =1.49), and the lowest number in winter (6, 2.5%), as summarized in Table 3.

Table 3. Seasonal prevalence of *C. jejuni* isolated from poultry meat sold in Yemen

Seasons	Total samples	Positive No. (%)	Negative No. (%)	95% CI (OR)	P-value
Winter	236	6 (2.5)	230 (97.5)	NA 1.00 Ref.	NA
Spring	366	9 (2.5)	357 (93.5)	0.34–2.74 0.96	0.945
Summer	347	13 (3.7)	334 (96.3)	0.56–3.98 1.49	0.422
Autumn	251	12 (4.8)	239 (95.2)	0.70–5.28 1.92	0.203

95% CI: 95% Confidence Interval; **OR =** Odds Ratio; ***P* value**: Probability value <0.05 (significant), **Ref.** = Reference, **NA** = Not applicable

Antimicrobial susceptibility test

This finding revealed that all *C. jejuni* isolates were completely resistant to macrolide antibiotics (erythromycin and azithromycin) and highly resistant to aminoglycosides, with 100% resistance against streptomycin and 85.0% resistance against gentamicin. All isolated *C. jejuni* were completely resistant to clindamycin. However, all *C. jejuni* isolates showed complete sensitivity (100%) to ampicillin and chloramphenicol, 90.0% to ciprofloxacin, 85.0% to tetracycline, and 80% to nalidixic acid, as listed in Table (4).

Table 4. Antimicrobial susceptibility pattern of *C. jejuni* isolated from poultry meat

Families of antibiotics	Antibiotic used	Disk Content (µg)	Inhabitation zone range (mm) Mean ±SD	Sensitive No. (%)	Resistance No. (%)
β-Lactams	**Ampicillin**	10	18.0–32.0 (25.7±3.79)	40 (100.0)	0 (0.0)
Phenicols	**Chloramphenicol**	30	3.0–32.9 (26.9±5.95)	40 (100.0)	0 (0.0)
Lincosamides	**Clindamycin**	2	0.0–0.0	0 (0.0)	40 (100.0)
Macrolides	**Erythromycin**	15	0.0–11.0	0 (0.0)	40 (100.0)

			(1.17±3.011)		
	Azithromycin	15	0.0–28.0 (8.80±6.49)	0 (0.0)	40 (100.0)
Aminoglycosides	**Gentamicin**	10	0.0–22.0 (9.35±4.63)	6 (15.0)	34 (85.0)
	Streptomycin	10	0.0–1.6 (0.18±0.450)	0 (0.0)	40 (100.0)
Tetracycline	**Tetracycline**	30	0.0–29.0 (18.90±6.32)	34 (85.0)	6 (15.0)
Quinolone	**Nalidixic acid**	30	3.0–19.12 (19.12±8.03)	32 (80.0)	8 (20.0)
Fluoroquinolones	**Ciprofloxacin**	5	8.0–31.0 (25.32±5.53)	36 (90.0)	4 (10.0)

µg = microgram; mm = Millimeter, SD = Standard Deviation

Multi-Drug Resistance Pattern

Table 5 shows that all *C. jejuni* isolates (40, 100%) had a multidrug resistance (MDR) profile against different antimicrobial classes. More than half of the *C. jejuni* isolates were resistant to five antimicrobial agents (24, 60.0%), and 23 (57.5%) were resistant to azithromycin, clindamycin, erythromycin, gentamicin, and streptomycin. Additionally, ten (25.0%) *C. jejuni* isolates were resistant to six different antimicrobial agents, with the majority of these isolates (4, 10.0%) exhibiting resistance to azithromycin, clindamycin, erythromycin, gentamicin, nalidixic acid, and streptomycin. In addition, four *C. jejuni* isolates were resistant to four antimicrobial agents (10%), and two were resistant to seven (5%).

Table 5. Prevalence of MDR among *C. jejuni* strains isolated from poultry meat samples

Number of MRD	Multi-resistance profile	Resistance No. (%)	Total No. (%)
Four	AZM–CD–E–S	2 (5.0)	4 (10.0)
	AZM–CD–E–GEN–S	2 (5.0)	
Five	AZM–CD–E–NA–S	1 (2.5)	24 (60.0)
	AZM–CD–E–GEN–S	23 (57.5)	
Six	AZM–CD–E–GEN–NA–S	4 (10.0)	10 (25.0)
	AZM–CIP–CD–E–GEN–S	2 (5.0)	
	AZM–CIP–CD–E–S–TE	2 (5.0)	
	AZM–CD–E–GEN–S–TE	2 (5.0)	

Seven	AZM–CD–E–GEN–NA–S–TE	2 (5.0)	2 (5.0)

AZM= Azithromycin, CIP= Ciprofloxacin, CD= Clindamycin, E= Erythromycin, GEN= Gentamicin, NA= Nalidixic acid, S= Streptomycin, TE= Tetracycline.

Discussion

The results of this study revealed that the overall prevalence of *C. jejuni* isolated from poultry meat was 12.12%, which is significantly lower than that reported in previous studies that revealed the prevalence of *C. jejuni* at 13.7–17.2% in China [31–32], 68.7% in Australia [32], 57% in Pakistan [34], 26.4% in Saudi Arabia [24], 17% in Iraq [16], and between 16% and 29.3% in Morocco [17, 35]. The difference between our results and those of previous studies may be due to the sample size, geographical area, diagnostic techniques used, and policy implementation in some countries. Undercooked meat consumption is not the only concern regarding the transmission of these bacteria to humans; however, accidental contamination of raw meat during cooking can spread to humans and other food. This situation highlights the need for public awareness about how pathogens can be transmitted through meat, as well as the importance of proper meat handling to prevent such transmission.

This study's findings showed that local poultry meat has significantly higher levels of *C. jejuni* bacteria than imported poultry meat. Previous reports [16, 36] have well documented the higher prevalence of *C. jejuni* in local poultry meat compared to imported poultry. This high contamination rate in local poultry meat is due to inadequate hygiene in slaughterhouses and fresh poultry shops and a lack of training for the workers. Therefore, mandatory training programs should be implemented for slaughterhouse and fresh poultry shop workers to ensure the provision of safe food for the community. Furthermore, earlier reports have shown that imported poultry meat was contaminated with *C. jejuni* [12, 36–37]. A recent report from Estonia suggests that imported poultry meat may be a potential source of *Campylobacter* infection in humans, based on a genetic comparison of *Campylobacter* isolates from humans and poultry meat [12]. This illustrates the potential risk of imported foodborne pathogens, particularly antibiotic-resistant ones, and their impact on public health. This necessitates stricter controls on imported meat and laboratory testing, particularly for bacteriological purposes, before they are permitted to be sold in the market.

This finding revealed that the highest proportion of *C. jejuni* was detected in meat samples taken from the intestines, and this result is supported by previous studies [10–11, 38]. The intestinal tract of chickens, particularly the colon and caecum, which are thought to be areas of tropism for many *Campylobacter* species, may be the cause of this prevalence [39]. Furthermore, these results indicate that samples collected from poultry skin had the second highest level of contamination with *C. jejuni* bacteria, which is consistent with many previous studies [17, 38, 40]. The presence of *C. jejuni* in poultry skin samples is attributed to the bacteria being transmitted through fecal contamination during poultry farming on farms. Furthermore, this bacterium can be transmitted from the intestinal dissection to the rest of the poultry body parts during slaughter and processing in slaughterhouses [41]. Regular inspections of slaughterhouses and fresh poultry shops should be conducted to avoid health risks associated with meat-borne pathogens.

Logistic analysis did not show the effect of seasonal variations on C. *jejuni* prevalence rates, indicating that the bacteria are widespread throughout the year in the study area without strong seasonal preference. These results are fully consistent with previous studies conducted in European Union countries [9], Morocco [35], Tunisia [42]. Several previous studies have documented a strong relationship between changes in temperature and rainfall and increased *Campylobacter* infections in humans [43–45]. Rising temperatures provide a favorable environment for the growth and multiplication of bacteria and their transmission via vectors. Moreover, human activity increases during warmer seasons, which also contributes to increased transmission of these bacteria during warmer seasons [45]. To develop an effective strategy to limit the spread of infection, studies should be conducted to explore the environmental and behavioral factors associated with the seasonal spread of *Campylobacter* bacteria.

Comparative analysis of 16S RNA sequences revealed a high degree of genetic similarity, exceeding 99%, between *C. jejuni* isolates from local meat, forming a distinct local genetic cluster. They shared the same genotype at most loci, indicating that the local isolates shared a common evolutionary origin and belonged to a single local genetic cluster. In contrast, the *C. jejuni* isolate from imported meat showed numerous nucleotide differences compared with the local strains, suggesting a distinct lineage.

All *C. jejuni* isolates in this study were susceptible to ampicillin, which is consistent with the study by Kouglenou et al. [36] and differs from the study by Hagos et al. [46]. None of the *C. jejuni* isolates showed resistance to chloramphenicol, and this finding is in line with the other studies [40, 46–47]. This result indicated that *C. jejuni* was completely resistant to clindamycin, in contrast to the findings of Owiredu et al. [33], which showed that all isolates of *C. jejuni* were sensitive to this antibiotic.

These results show that *C. jejuni* was completely resistant to macrolide antibiotics, and this study is similar to other reports [24, 35, 48]. The results of this study are much higher than those recorded in previous studies in various countries of the world, as it was found that *C. jejuni* isolates from poultry meat were resistant to the antibiotic erythromycin, ranging from 1.6% to 19.2% in China [31–32, 49], 50% in Brazil [50], 29% in Morocco [17], 20% in Pakistan [34], 2.8% in Romania [40], and 56.6% in Thailand [51].

Previous studies have shown that *Campylobacter* resistance to macrolide antibiotics results from point mutations occurring at positions 2074 and 2075 in the V domain of 23S rRNA [52–53]. The emergence of macrolide-resistant strains of *C. jejuni* is alarming, given that these antibiotics are considered the first-line treatment for *Campylobacter* infections. Therefore, it is important to strengthen surveillance systems to regularly monitor macrolide resistance in meat and other foods, as well as to limit the use of these antibiotics in poultry farming.

The present finding found a high rate of *C. jejuni* isolates resistant to gentamicin, and this is in agreement with previous reports [17, 54]. In contrast, the lower rate of *C. jejuni* resistance to gentamicin was reported at 35.5% in Tianjin [49], 15.4% in Jordan [16]**,** and 12.0% in Morocco [35]**.** Furthermore, all *C. jejuni* isolates were found to be resistant to streptomycin, which is inconsistent with the results of Hagos et al. [46] and Wieczorek et al. [31].

The resistance of *Campylobacter* to gentamicin is attributed to the presence of resistance genes, which the bacteria may acquire as a result of the overuse of antibiotics during animal production. These genes include *aacA4, aacA/aphD*, which encodes aminoglycoside-modifying enzymes (AMEs), enhancing the bacteria's resistance to gentamicin. Furthermore, gentamicin resistance genes such as pCG8245 are often found on plasmids or transposons in some bacterial strains and are readily transferred between them via horizontal gene transfer. Some bacterial strains possess pumping systems that enable them to expel certain antibiotics from the cell [50-51].

The lower resistance of *C. jejuni* isolates was reported to tetracycline, and this finding is consistent with earlier reports [33, 40]. In different reports, the resistance rate of *C. jejuni* isolates for poultry meat was highly recorded at 100% [35], 77.4% [49], and 71.4% [36]**.** Furthermore, 10% of *C. jejuni* isolates were found to be resistant to ciprofloxacin, which is significantly lower than the 24.4% recently documented by Owiredu et al. [33]. Conversely, these results are in distinct contrast to previous studies that reported high rates of *C. jejuni* resistance to ciprofloxacin, ranging from 77% to 100% [31, 35, 49]. These results demonstrate the value of implementing prudent policies regarding the use of antimicrobials in the Yemeni food production chain, as food may serve as a source of infection for humans with antimicrobial-resistant microbes.

These outcomes observed that all *C. jejuni* isolates were resistant to multiple antibiotics, and more than half were resistant to five types of antibiotics, including azithromycin, clindamycin, erythromycin, gentamicin, and streptomycin. The high rates of multidrug resistance of *Campylobacter* bacteria were previously reported at 100% in Egypt [16], 96.6% in Algeria [14], 90% in South Africa [15], 69% in Poland [57], 68% in Morocco [17], 55.8% in West Africa [36], and 55% in Jordan [48].

The emergence of *C. jejuni* strains that are resistant to multi-antibiotics is a concern for human health and the poultry industry, and these strains may be attributed to the misuse and long-term use of antibiotics in poultry production, particularly in Yemen, due to the absence of protocols regarding regulating their use [58–61]. Therefore, it is essential to implement a One Health approach that involves multiple strategies to mitigate the build-up of antimicrobial resistance in the food supply and reduce unnecessary antibiotic use in Yemeni poultry. A multifaceted strategy that incorporates stronger food safety regulations, more intelligent antibiotic use, and improved biosecurity measures is required to apply the "One Health" framework to poultry farming [62]. Additionally, increasing surveillance in slaughterhouses, retail establishments, and medical facilities can achieve long-term protection for humans and animals by early identification of resistant strains.

Strength and limitation of this study

The use of molecular analysis techniques, which are the gold standard for detecting *C. jejuni* in poultry meat samples, is a major strength of this study. The results of this study will significantly contribute to bridging the gap regarding the prevalence of *C. jejuni* in poultry meat sold in the study area, which has not been covered in previous studies. Furthermore, these results provide a baseline database for future studies on foodborne pathogens and their antibiotic resistance. These outcomes are valuable for healthcare decision-makers to develop foodborne disease surveillance programs and preventive interventions to reduce the spread of such diseases. Despite the importance of this study, it has some limitations. This study did not address the detection of *C. jejuni* serotypes in poultry meat because of limited resources. Furthermore, it did not aim to identify the pathogens or genes contributing to antibiotic resistance. Additionally, this investigation did not address the environmental factors that contribute to the prevalence of *C. jejuni* in the study area. Moreover, the current study did not evaluate the knowledge, practices, and attitudes of butchers working in these facilities regarding foodborne diseases. Due to limited resources in Yemen, antibiotic susceptibility testing was conducted using the disk diffusion method, which is not the gold standard. Additionally, reference strains of *C. jejuni* were unavailable as controls for testing because of economic conditions, armed conflict, and logistical constraints.

Conclusion

This study indicates that the high prevalence of *C. jejuni* in poultry meat poses a serious threat to human health in the study area. Locally produced poultry meat contains *C. jejuni* six times more than imported poultry, which is a serious concern if strict controls are not implemented in local poultry slaughterhouses and meat markets. The high prevalence of *C. jejuni* in the intestines and skin necessitates careful handling during poultry preparation to prevent the spread of pathogens to other meat parts. The high resistance of *C. jejuni* isolates to first-line antibiotics poses a considerable public health threat, as it limits the treatment alternatives available to physicians for severe infections. Given the proliferation of multidrug-resistant strains, the use of macrolides as a first-line treatment in the region and the updating of alternative treatment protocols should be urgently reviewed. Therefore, it is essential to immediately initiate follow-up studies linking human and animal *C. jejuni* strains to implement effective strategies for controlling transmission before it develops into difficult-to-contain epidemics. Further studies to identify serotypes, virulence genes, and antibiotic resistance genes in *C.*

jejuni are crucial for understanding the epidemiology of this bacterium and its genetic changes. Enhancing the knowledge and practices of slaughterhouse workers is a practical and important step in implementing meat-borne disease control programs and improving public health in the country. Strict monitoring of slaughterhouses and import points, particularly during peak seasons, is critical for preventing outbreaks of multidrug-resistant strains transmitted through meat.

Abbreviation

bp: Base Pair
EUCAST: European Committee on Antimicrobial Susceptibility Testing
CFU: Colony Forming Unit
ISO: International Organization for Standardization
MDR: Multidrug Resistance
MH-F: Mueller-Hinton Fastidious Agar
NCBI: National Center for Biotechnology Information
OR: Odds Ratio
P value: Probability value <0.05 (significant)
PCR: Polymerase Chain Reaction
rRNA: Ribosomal ribonucleic acid
SPSS: Statistical Package for Social Sciences.
χ^2: Chi square
95% CI: 95% Confidence Interval

Declarations

Ethics statement and consent to participate

This study was approved by the Ethics Committee of the Deanship of Postgraduate Studies and Scientific Research, Sana'a University, No. (92), and dated (10/10/2022). Postmortem poultry meat samples were used, and this study did not include any experiments on live poultry or interventions in the natural environment. Verbal consent was obtained from the vendors while maintaining the confidentiality of the data and sources of the samples and not disclosing any information related to the vendors' identities. Furthermore, local poultry meat samples were collected from retail slaughterhouses, while imported poultry meat samples were collected from supermarkets. Additionally, all biosafety and environmental protection guidelines were adhered to during sample collection, transportation, and analysis, in accordance with [25].

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request. The 16S rRNA gene sequences have been deposited in GenBank under the accession numbers PV263606.1, PV263587.1, and PX392582.1.

Consent for publication

Not applicable.

Author's contribution

Al-Bana M: collected data and samples, conducted the experiments, processed the data, interpreted the data, and wrote the original draft. **Alghalibi S**: conceived the study, supervised it, interpreted the findings, and reviewed and edited the manuscript. **Abdullah Q**: assisted in study design and supervision, interpreted the findings, and reviewed the manuscript. **Edrees W**: assisted in processing and analyzing the data, interpreted the data, wrote the original draft, and edited the manuscript. **Al-Shehari W**: Contributed to data analysis and interpretation and reviewed and edited the manuscript. **Jaml N**: Assisted in conducting the molecular experiments and processed and interpreted the data. **Al-Arnoot S**: Contributed to processing the data, analyzing and interpreting it, and writing the original draft. **Al-Thobhani A**: assisted in conducting the experiments, collected and analyzed data, and interpreted the findings. **Al-Akhali B**: contributed to collecting samples and conducted the experiments. All authors reviewed the manuscript and approved the submitted version.

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