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The risks of Campylobacter in broiler meat and table eggs, the environmental context surrounding meat processing in small-scale slaughterhouses, and restaurants in correlation to patients with gastroenteritis

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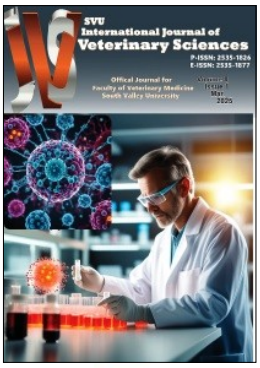

ABSTRACT

Poultry is the most substantial contributor to human campylobacteriosis presenting a great challenge to food safety. This study evaluated the risks of Campylobacter contamination during various stages of slaughter, and potential cross-contamination scenarios at slaughterhouses and restaurants. 460 samples were collected from the Sohag governorate, including chicken meat, stool from persons with gastroenteritis, and environmental samples (120 for each), in addition to 100 table eggs (eggshell, and egg content). Samples underwent bacteriological analysis, and the isolates were confirmed by multiplex PCR for the 23S rRNA, hip O, and gly A genes. Campylobacter prevalence rates in broiler meat, table eggs, environment, and human stool samples determined by multiplex PCR were 9.17, 2, 7.5, and 6%, respectively, with overall positive samples of 6.3% (28/460). 75% (21/28) of the isolates were Campylobacter jejuni, 25% (7/28) were Campylobacter coli, and 1 isolate had mixed contamination. Poultry fecal matter, broiler meat, and table eggs could be a high risk of Campylobacter to humans, highlighting the need for targeted interventions in the poultry, and egg industry to mitigate the risk of Campylobacter infections. Improved food handling practices at restaurant and house kitchens are essential to reduce contamination.

Keywords*: Chicken meat, Campylobacter, Environment, gastroenteritis, Multiplex PCR*

INTRODUCTION

Campylobacter (mainly C. jejuni and, to a lesser degree, C. coli) ranks among the four principal etiological agents of gastroenteritis globally with increased prevalence in both developed and developing nations over the past decade (Costa and Iraola, 2019). Campylobacteriosis represents a considerable global public health dilemma, and the most frequently reported zoonotic disease in the European Union (EU) since 2005 (Kaakoush et al., 2015b; WHO,2020). In 2020, the confirmed cases of campylobacteriosis reached 120,946, with a 40.3 per 100,000 EU notification rate (EFSA-ECDC, 2021). In the United States, approximately 1.5 million Campylobacter infections are reported annually, with 20–30% of these cases being associated with the consumption of poultry meat (CDC, 2018, 2019).

Campylobacteriosis manifests sporadic cases of gastroenteritis or as minor outbreaks that typically develop after 24 to 72 hours as an incubation period,

present with diarrhea, fever, abdominal discomfort, nausea, malaise, headaches, and cramps, which are self-limiting and resolve within 24–48 hours in most cases, however, a protracted course may occur (Fitzgerald, 2015). In instances of severe disease forms, dehydration, bacteremia, or sepsis symptoms resembling ulcerative colitis or acute appendicitis may occur. Diarrheal illness may exhibit heightened severity in young children (Belina et al., 2024). 36% of persons are at risk of subsequent health complications 1–2 years of acute Campylobacter infections. The irritable bowel syndrome (IBS) may occur in 9-13% of C. jejuni infection (Pimentel et al., 2015; Geissler et al., 2017), and reactive arthritis (2–5% of patients). While, Guillain–Barré syndrome (0.1% of patients), often results in neuromuscular paralysis which may cause permanent nerve damage in some cases, (Scallan et al., 2015; CDC, 2018).

The chicken was the predominant source of campylobacteriosis cases in Denmark (45.8- 65.4%), the United States (68–72%), Japan, and Australia (over 80%) (Vetchapitak and Misawa, 2019; Pascoe, 2022; Brinch et al., 2023; McLure et al., 2023). 31–66% of Campylobacter jejuni (C. jejuni) clinical isolates were related to chickens as the principal reservoirs, with raw or inadequately cooked poultry meat, and meat products contributing to approximately 25% of Campylobacteriosis cases (Thépault et al., 2018; Clarke and Ajlouni, 2021). Fecal leaks during evisceration, contact with contaminated equipment, and exposure to contaminated water substantially elevate the risk of contamination. For instance, the discharge of merely 5 mg of cecal matter during evisceration augments Campylobacter loads by 0.6 log CFU in prechill rinses (Pascoe et al., 2023).

The gastrointestinal tract of chicken functions as a natural reservoir for Campylobacter, thereby facilitating its propagation throughout eggshells (EFSA-ECDC, 2021).

Consequently, this work aimed to scrutinize the risks of Campylobacter in broiler meat and table eggs, the environmental context surrounding meat processing in small-scale slaughterhouses, and restaurants in correlation to patients with gastroenteritis.

MATERIALS AND METHODS

Sample collection

A total of 460 specimens were analyzed, comprising 120 specimens derived from broiler meat and chicken meat products, 100 table eggs, and 120 human fecal samples obtained from healthcare facilities and clinical laboratories, along with 120 specimens sourced from poultry processing environments, specifically poultry slaughter shops,

Table A: Primer sequences of Campylobacter spp. according to Wang (2002)

Target gene		Primer	Oligonucleotide sequence (5'→ 3')	Product size (bp)
23S	F	*23S rRNA* (All Spp.)	TATACCGGTAAGGAGTGCTGGAG	650 bp
	R		ATCAATTAACCTTCGAGCACCG	
hip O	F	CJ (*C. jejuni*)	ACTTCTTTATTGCTTGCTGC	323 bp
	R		GCCACAACAAGTAAAGAAGC	
gly A	F	CC (*C. coli*)	GTAAAACCAAAGCTTATCGTG	126 bp
	R		TCCAGCAATGTGTGCAATG	

Table B: PCR Cycling conditions according to Wang et al. (2002)

No. of cycles	Time	Temp.	Step
1 cycle	6 min.	94˚C	1. Primary denaturation and activation of hot start green Taq DNA polymerase.
2. Cycling			
35 cycles	30 sec.	95˚C	A. Secondary denaturation
	30 sec.	59˚C	B. Primer annealing
	30 sec.	72˚C	C. Extension
1 cycle	7 min.	72˚C	3. Final extension

and restaurant kitchens. Each specimen was collected in a sterile polyethylene bag and transported to the laboratory (Animal Health Research Institute, Sohag Sub-Laboratory) utilizing a sampling box equipped with ice pads, ensuring minimal delay (Donnison, 2003).

Sample Preparation

Twenty-five grams of each chicken meat specimen was aseptically transferred to a sterile stomacher bag containing 225 ml of Bolton broth with 5% Laked horse blood (Oxoid, SR048) and modified Bolton broth selective supplement (Oxoid, SR0183E), then homogenized for 1 minute (Hunt and Abeyta, 1998). Egg shells and their contents were prepared according to Jones and Musgrove (2007) with modifications pooled samples included 2 eggs from each package. Swabs from human fecal matter and poultry droppings were collected in sterile containers containing 9 ml of Bolton broth with additional supplementation. Surface swabs were procured using a 25 cm^2 sterile metal template. Water samples were gathered in clean, sterile 500 ml bottles and subsequently concentrated through centrifugation at 20,000 rpm for 10 minutes. The resultant pellet was resuspended in test tubes containing 9 ml of Bolton broth supplemented with water (ISO 17995:2019).

Isolation and Identification

The specimens underwent aseptic incubation for 4 hours at 37°C to facilitate the resuscitation of stressed organisms, followed by subsequent incubation at 42°C for a period ranging from 20 to 44 hours under micro-aerophilic conditions obtained by a gas generating kit (Oxoid CampyGen, CN0035A). Environmental swabs were immersed in sterile tubes containing 10 ml of Bolton broth (Hunt and Abeyta, 1998). A loopful (10 µl) was extracted from each Bolton broth enrichment culture after 48 hours and streaked onto Modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) (Oxoid, CM0739) with selective supplements (Oxoid, SR0155E). The inoculated plates were incubated microaerobically in an anaerobic jar (Oxoid), and gas-generating kits at 42°C for 48 hours (Bolton et al., 1984). Human stool and poultry fecal swabs were inoculated directly onto mCCDA (Maher et al., 2003).

The confirmation of positive strains was accomplished through Gram staining, motility assessment, oxidase tests (Oxoid, MB0266A), catalase reaction, Hippurate hydrolysis (Sodium Hippurate, Sigma, H9380; Ninhydrin, Merck, 6762), H2S reaction on triple sugar iron agar slants (Oxoid, CM0277), susceptibility testing to nalidixic acid (30 µg) and cephalothin (30 µg) on Muller Hinton agar (Oxoid, CM0337) (WOAH, 2005; ISO, 2006).

Molecular Characterization

Bacterial DNA was extracted via a Wizard® Genomic DNA Purification Kit (Promega, A1120). Primer sequences (Metabion, Germany) of 23S rRNA, hip O, and gly A genes of Campylobacter species are shown in Table A. In the reaction tube, 2.5 µl of Green Taq hot-start polymerase, 1 µl of each primer, 0.5 µl of DNA template, and 20 µl nuclease-free water were vortexed. Cycling included 94°C for 6 minutes as an initial denaturation step, followed by 30 cycles (denaturation at 95°C for 30 seconds, annealing at 59°C for 30 seconds, and extension at 72°C for 30 seconds for species genes), ending with a final extension for 7 minutes at 72°C using a thermocycler (Takara, Code No. RR310A) according to Wang et al. (2002) as shown in Table B. The PCR products were electrophorized via 1.5% agarose gel (Sambrook and Russell, 2001), stained with ethidium bromide, and visualized on a UV transilluminator (Andrzejewska et al., 2011).

Statistical analysis

The data were statistically analyzed via SPSS version 22, and all significance levels were considered at $P < 0.05$. The associations between positive campylobacteriosis and the sources of samples were calculated via Pearson's chi-square test. The model was built in @Risk 7.5.2 (Palisade, Inc.) software, and a Monte Carlo simulation (10,000 iterations) was used to estimate the probability of risks using Python software (Zio, 2013).

RESULTS

Positive colonies displayed phenotypic traits consistent with a grayish-white coloration, convex morphology, and a moist texture. The organisms were identified as Gram-negative, spiral, S-shaped, and curved rod-shaped bacteria, exhibiting a corkscrew-like movement, with positive results for catalase and oxidase tests (C. jejuni/C. coli). Species were initially

Table 1: Incidence of Campylobacter species in broiler meat, table eggs, and environmental samples via biochemical tests

Samples	Presumptive culture			Biochemical identification		Hippurate			
	N	Positive	%	Positive	%	Positive	%	Negative	%
Broiler meat									
Carcass	30	12	40.0	6	20	3	13.33	2	6.67
Breast	30	5	16.67	2	6.7	2	8.89	0	0
Thigh	30	8	26.67	4	10	4	15.56	2	0
Products	30	0	0	-	-	-	-	-	-
	120	25	20.83	12	10	9	7.5	4	**3.33**
Table egg									
Egg content	50	0	0	-	-	-	-	-	-
Eggshell	50	2	4	2	4	2	4	1	2
	100	2	2	2	2	2	2	1	**1**
Environment									
Slaughter shops	30	14	46.67	7	23.3	7	23.3	0	0
Restaurant swabs	30	5	16.67	3	10	3	10	0	0
Fecal swabs	30	15	50	10	33.3	3	10	2	3.33
Water	30	0	0	-		-	-	-	-
	120	34	28.33	20	16.67	13	10.83	2	1.67

-: not detected in culture and biochemical. Different superscript letters indicate significant differences. There was a significant relationship [p-value=0.0053] between age group and presumptive positivity for Campylobacter. The incidence rates are not evenly distributed across age groups.

Table 2: Incidence of Campylobacter species in human stool samples via biochemical tests

Samples	Presumptive culture			Biochemical identification		Hippurate			
		Positive	%	Positive	%	Positive	%	Negative	%
Children's diarrhea									
0.5 - 4 years	30	14	46.67[a]	7	23.3	3	10	1	3.3
5 -15 years	30	10	33.33[a]	5	16.7	1	6.67	1	3.3
16-35 years	30	4	13.33[b]	2	6.7	-	-	-	-
36-60 years	30	0	0[b]	-	-	-	-	-	-
	120	28	23.33	14	11.67	4	3.33	2	1.67

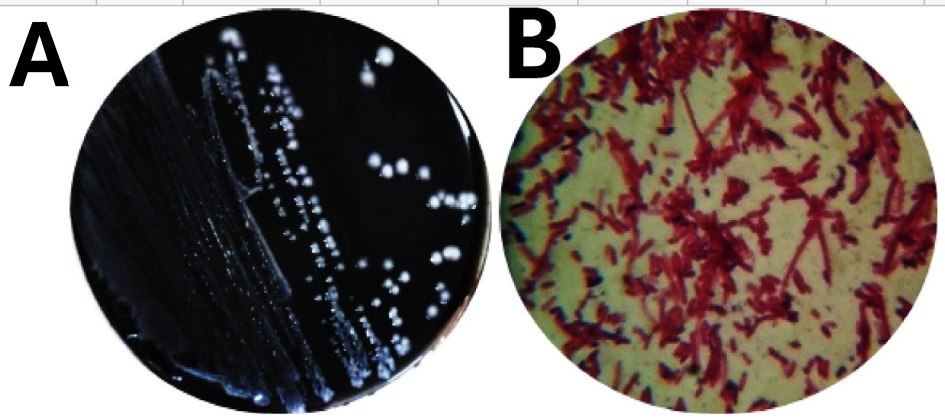

Figure1: (A) Campylobacter colonies on mCCDA plate. (B) microscopic appearance and staining reaction of Campylobacter spp. Showing Gram-negative bacilli, curved rods, spiral-shaped, resembling a seagull wing, and short rods.

Table 3: Prevalence of *C. jejuni* and *C. coli* in chicken meat, table eggs, and environmental samples determined via multiplex PCR.

Samples	23S rRNA			C. jejuni		C. coli	
		No.	%	No	%	No.	%
Chicken meat							
Broiler carcass	30	5	16.67	3	10	2	6.67
Breast	30	2	6.67	2	6.67	0	0
Thigh	30	4	13.33	3	10	1	0
Products	30	-	-	-	-	-	-
	120	**11**	**9.17**	**8**	**6.67**	**3**	**2.5**
Table egg							
Egg content	50	0	0	-	-	-	-
Eggshell	50	**2**	**4**	2	4	1*	2
	100	**2**	**2**	**2**	**2**	**1***	**1**
Environmental sample							
Slaughter shop	30	3	10	3	10	0	0
Restaurant swabs	30	1	3.33	1	3.33	0	0
Cloacal swabs*	30	5	16.67	3	10	2	6.67
Water	30	-	-	-	-	-	-
	120	**9**	**7.5**	**7**	**5.83**	**2**	**2.22**
Total	**340**	**22**	**6.47**	**17**	**5**	**6**	**1.76**

Table 4: Prevalence of C. jejuni and C. coli in human stool samples determined via multiplex PCR.

Samples	23S rRNA			C. jejuni		C. coli	
		No.	%	No	%	No.	%
Human							
0.5 - 4 years	30	4	13.33	3	10	1	3.33
5 – 15 years	30	2	6.67	1	6.67	1	0
16-35 years	30	-	-	-	-	-	-
36-60 years	30	-	-	-	-	-	-
	120	**6**	**5**	**4**	3.33	**2**	1.67

-: not detected in culture or biochemical.

categorized based on their capacity to hydrolyze Hippurate, susceptibility to cephalothin and nalidixic acid, and H2S production as delineated by ISO10272-1, 2006) (Figure 1). Campylobacter was identified in chicken meat, table eggs, environment, and human fecal samples at prevalence rates of 20.83, 3, 28.33, and 23.33%, respectively. The prevalence rates among chicken carcasses, breasts, and thighs were 40, 16.67, and 26.67%, respectively, whereas it was undetectable in processed chicken Luncheon and chicken nuggets. 46.67, 16.67, and 50% of slaughterhouses, restaurant swabs, and fecal samples, respectively were contaminated. While tap water samples were free of Campylobacter (Table 1).

Campylobacter was found in 46.67% (14/30) of children aged from 6 months to 5 years, 33.33% (10/30) in those aged 6–15 years, and 13.33% (4/30) in those aged 16–35 years; however, no detection was reported in patients aged 36–60 years (Table 2).

Multiplex PCR of the 23S rRNA gene in 9.17% (11/120), 2% (2/100), 7.5% (9/120), and 5% (6/120) of the chicken meat samples, eggs, environment, and human samples (Tables 3,4 and Figures 2-4). Genetic analysis of positive isolates for the differentiation of Campylobacter species through detection of the hipO and glyA genes revealed that 4.57% (21/460) were C. jejuni [21/28, 75%], 1.74% (8/460) [7/28, 25%] were C. coli, and 1 sample was mixed contaminated with C.

Table 5: Correlation of C. jejuni and C. coli from broiler meat, egg, and environment to human infection.

Samples	23S rRNA			C. jejuni		C. coli		mixed		
		No.	%	No	%	No.	%	No.	%	OR [95% CI]
Chicken meat	120	11	9.17	8	6.67	3	1.67	0	0	1.071 [0.15:7.62]
Table egg	100	2	2	2	2	1	1	1	1	0.5 [0.067:3.764)
Environment	120	9	7.5	7	4	2	2	0	0	0.5 [0.067:3.764)
Human	120	6	5	4	3.33	2	1.67	0	0	1.333 [0.123:14.336]
Total	**460**	**28**	**6.09**	**21**	**4.57**	**8**	**1.74**	**1**	**0.22**	$r \approx 0.852$
C. jejuni/C. coli ratio (n=28)				**21**	**75**	**7**	**25**	**1**	**3.57**	

Odds Ratio (OR = 1.73): People are 1.73 times more likely to test positive for Campylobacter spp. if chicken samples are contaminated, though the CI includes 1, indicating this is not statistically significant. 95% CI (0.61–4.93): The wide CI suggests limited precision. The Pearson correlation coefficient $r \approx 0.852$, indicates a strong positive correlation between C. jejuni and C. coli across the samples. Chicken Meat, OR = 1.071, and the Z-value for a 95% confidence interval is 1.96.

Table 6: Probability of high risk of *C. jejuni* and *C. coli* in the examined samples (%).

	C. jejuni	*C. coli*
Chicken meat	74.51	49.28
Table egg	12.23	1.21
Environmental sample	49.22	15.72
Human	15.38	2.42

A Monte Carlo simulation with a Latin hypercube sampling method (10,000 iterations) was used.

jejuni and C.coli.

People are 1.73 times [the odds ratio (OR= 1.73)] more likely to test positive for Campylobacter spp. if chicken samples were contaminated. A strong positive correlation between C. jejuni and C. coli across the samples was indicated by the Pearson correlation coefficient $r \approx 0.852$. The OR of broiler meat was 1.071, and the Z-value for a 95% confidence interval was 1.96 (Table 5).

A Monte Carlo simulation for risk assessment with 10,000 iterations showed that the probability of C. jejuni /C. coli being at a high-risk level in broiler meat was 74.51/ 49.28%, indicating a high likelihood of contamination with C. jejuni and C. coli in broiler meat, in food environmental samples was 49.22, and 15.72%, suggesting a moderate risk level concern in environment settings, and 15.38% for C. jejuni which is relatively low compared to C. coli (2.42%), which is

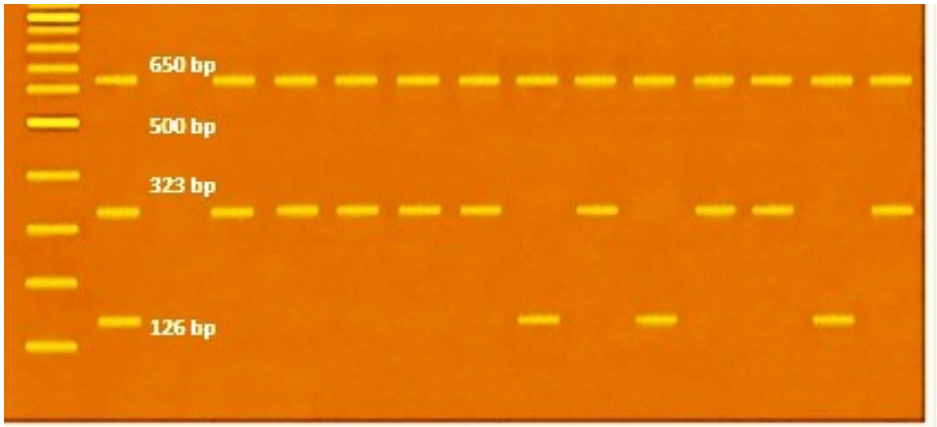

Figure 2: 1.5% agarose gel electrophoresis of m PCR of** Campylobacter **spp. Lane M: 100 bp DNA marker. Lane P: Control positive for** 23S rRNA **(650 bp)**, hip O **(323 bp), and** glyA **(126 bp) genes. Lane N: Control negative. Lanes from 1-5: Positive** Campylobacter **spp. for** 23S rRNA & C. jejuni hip O **gene from breaٹs and thighs**. **Lane 6: Positive** Campylobacter **spp. for** 23S rRNA & C. coli **for** glyA **gene from thigh. Lanes 7, 9, &10: Positive** Campylobacter **spp. for** 23S rRNA & C. jejuni hip O **gene from broiler carcasses. Lanes 8, & 11: Positive** Campylobacter **spp. for** 23S rRNA & C. coli gly A **gene from broiler carcasses. Lane 12: Positive** Campylobacter **spp. for** 23S rRNA & C. jejuni hip O **gene from reٹaurant.

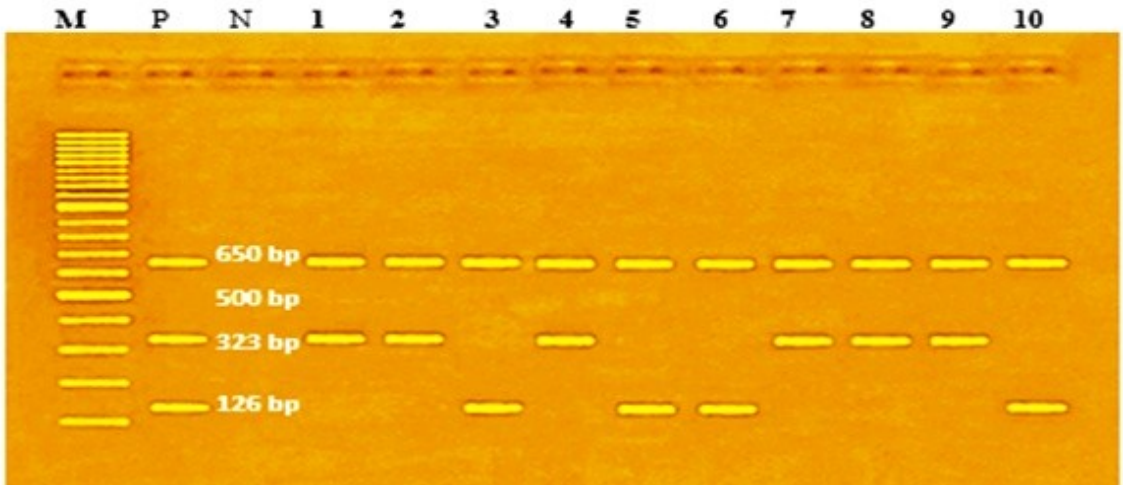

Figure 3: 1.5% agarose gel electrophoresis of mPCR for Campylobacter spp. Lane M: 100 bp DNA marker. Lane P: Control positive for 23S rRNA (650 bp), hipO (323 bp), and glyA (126 bp) genes. Lane N: Control negative. Lanes 1, & 2: Positive Campylobacter spp. for 23S rRNA & C. jejuni hip O gene from humans (>4 years). Lanes 3: Positive Campylobacter spp. for 23S rRNA & C. coli gly A gene from gene from humans (>4 years). Lane 5: Positive Campylobacter spp. for 23S rRNA & C. coli glyA gene from humans (>15 years). Lanes from 6, 10: Positive Campylobacter spp. for C. coli gly A gene from cloacal swabs. Lanes 7, 8, 9: Positive Campylobacter spp. for 23S rRNA & C. jejuni hip O gene from cloacal swabs.

quite low, indicating a very low risk of infection from C. coli in humans (Table 6).

DISCUSSION

Campylobacter is a Gram-negative, motile, non-sporulating bacterium, exhibiting a spiral or helical curved rod that can transition to filamentous or coccoid responding to environmental stresses (Kaakoush et al., 2015a). It is an obligate microaerophile possessing a relatively compact genome measuring 1.6 Mbp (Hakeem and Lu, 2021). Campylobacter species are the predominant etiological agent of gastroenteritis implicated in humans in the United Kingdom, with an annual incidence estimated at 300,000 cases (Holland and Mahmoudzadeh, 2020), chickens are the primary reservoir for thermotolerant Campylobacter spp., being accountable for an anticipated 80% of human Campylobacter infections (El-Gedawy et al., 2023).

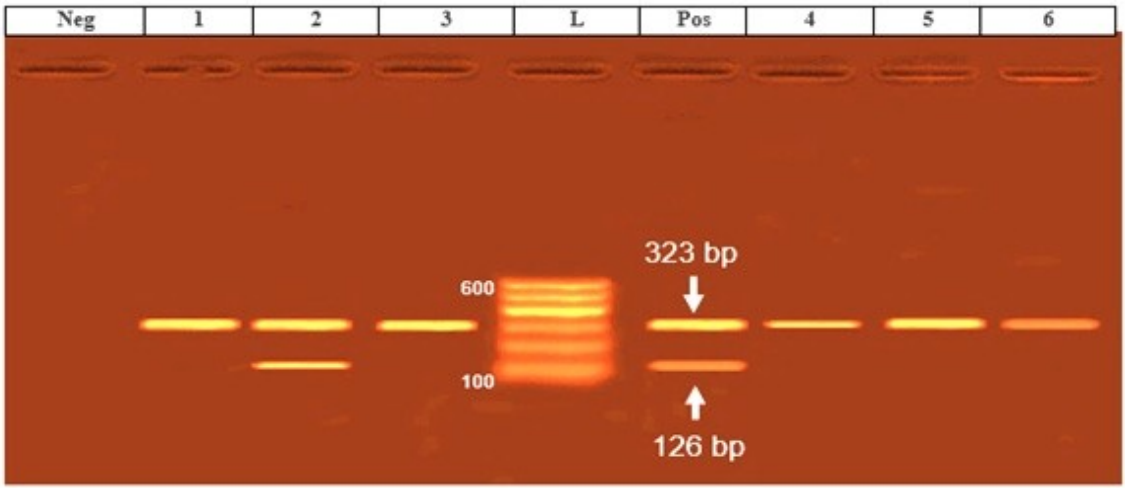

Figure 4: Agarose gel electrophoresis of diplex PCR for Campylobacter spp. Lane M: 100 bp DNA marker. Lane P: Control positive for hip O (323 bp), and gly A (126 bp) genes. Lane N: Control negative. Lane 1: Positive for C. jejuni hip O gene from human samples (>15 years). Lanes 2, and 3: Positive for C. coli gly A gene from eggshells. Lanes 4-6: Positive Campylobacter spp. for C. jejuni hip O gene from slaughter shops.

Table 1 demonstrates that 20.83% of the broiler meat and meat product samples tested positive for Campylobacter spp., as determined by culture and biochemical methodologies. This finding was similar to 20.7% in Italy (Nobile et al., 2013). This percentage is significantly lower than 66.7% in Brazil (Borges et al., 2020), 28.6% in the United Arab Emirates (Habib et al., 2022), and 77.41% in China (Lai et al., 2023). Variability in Campylobacter contamination rates in retail poultry meat was reported by Andrzejewska et al. (2015), documenting rates of 60.2, 45.9, 38.6, 29.3, and 32.0% from 2009 to 2012.

Campylobacter spp. was isolated from 12 out of 30 (40%) broiler carcasses (Table 1). A comparable result was documented by Andrzejewska et al. (2015), wherein the mean prevalence over five-years was calculated at 41.6%. This finding is lower than those reported by Bagherpour et al. (2014), Tang et al. (2020), and Bouhamed (2023), whose reported prevalence rates of 56.7%, 53.4%, and 53.33%, respectively. Lower results (33, and 13%) were reported by Yushina et al. (2020), and Chala et al. (2021), respectively.

Breast samples yielded a contamination rate of 16.67% (5/30) (Table 1), which aligns with the findings (16.67%) reported by Vashin and Stoyanchev (2004). This incidence was notably lower than the rates documented by Guyard-Nicodème et al. (2013), Awadallah et al. (2014), Sison et al. (2014), and Abd El-Tawab et al. (2015), who reported incidence rates of 47.9, 25.9, 47.5, and 30.8%, respectively.

Thigh samples demonstrated an isolation rate of 26.67% (Table 1), A higher rate (38.5%) was reported by Abd El-Tawab et al. (2015). Conversely, Gritti et al. (2011) could not isolate Campylobacter from thigh and breast samples by cultural and molecular techniques.

Chicken meat products, specifically chicken nuggets and chicken luncheons, exhibited a complete absence of Campylobacter spp., corroborating similar findings reported by Mohamad and Som (2012), in Alexandria, Samaha et al. (2012), Abdel-Malek (2015) in Assiut, and Lake and Cressey (2015) in New Zealand. The inability to isolate C. jejuni may be attributed to the elevated salt concentration and other constituents present in the marinades. C. jejuni is incapable of proliferation in environments containing ≥2% NaCl, yet it can survive in concentrations ranging from 0.5% to 1.5% NaCl (Gomes et al., 2018).

Campylobacter spp. could not be detected in the contents of table eggs, although 2/50 (4%) of eggshells were contaminated, and 2% as an overall rate in eggs (Table 1). These results are comparable to those obtained in Assiut City by Amin (2017). A higher incidence rate of Campylobacter in eggshells was reported at rates of 36% in Japan (Sato and Sashihara, 2010), 12% in Malaysia (Nor Faiza et al. (2013), and 25.6% in Tunisia Gharbi et al., (2022). Lower rates were reported by Hedawey and Yousef (2014).

The contamination rates of food environmental samples from slaughter shops, restaurants, and fecal swabs were 46.6%, 16.67%, and 50%, respectively with 28.33% (34/120) as an overall environmental contamination rate. The water samples were free of Campylobacter (Table 1). Slaughter shop samples were collected from the surfaces of the plucking machines, cutting boards, knives for eviscerating birds, and washing basins. Franchin et al. (2005) collected samples before slaughter, and Campylobacter spp. were found in litter, transport cages, cage rinse water, cloaca, feather, and breast support at rates of 37.5, 50, 25, 79.2, and 33.3%, respectively, with an average of 50%.

The restaurant samples focused on points where the raw chicken meat was washed, handled, and contacted surfaces. 16.67% of the restaurant samples were positive for Campylobacter spp., in contrast to Bellio et al. (2014) in Italy, who reported that all food contact surfaces were negative for Campylobacter.

Poultry fecal swabs had a 50% Campylobacter contamination rate, similar isolation rates were previously reported by Borges et al. (2020), Yushina et al. (2020), and Abdulwahab and Alhindwae (2025), who reported prevalence rates of 57, 50, and 44.5%, respectively. However, lower isolation rates of 35.9, 39.2, 38.1, and 35.1% were reported by Awadallah et al. (2014), Mäesaar et al. (2014), Torralbo et al. (2014), and Abd El?Tawab et al. (2015), respectively. Additionally, A lower result (1.9%) was obtained by Ghoneim et al. (2020) from cloacal swabs.

Campylobacter isolation rate varies in different studies due to various the type of examined samples, age and species of poultry, location, climate factors, hygienic measures, and isolation techniques (Kalupahana, et al., 2013; Chatur et al., 2014).

All water samples from slaughter shops and restaurants were free from Campylobacter spp. (Table 1), a similar result was reported by El Sayed (2016), and higher results (6.7, and 20.5%) were obtained in river water by Mubarak (2013), and Ghoneim et al. (2020).

Notably, Campylobacter spp. was identified in 23.33% of stool samples obtained from patients with enteritis through microbiological examination (Table 2), analogous results (27.55, and 29%) were reported in Assiut City by Abushahba et al. (2018) and Sayed et al. (2023). Also, Collado et al. (2013) documented rates of 28% and 25.7%, respectively. Nonetheless, lower incidence rates of 10, 2.6, 5.33. and 6.7% were observed by Salim et al. (2014), Vaishnavi et al. (2015), ElSayed (2016), and Ghoneim et al. (2020), respectively.

Campylobacter infection constitutes a significant etiological factor for diarrhea among pediatric populations in Egypt (Kaakoush et al., 2015a; Abdel-Ghany, 2019). Approximately 85% of children in Egypt experience infection with Campylobacter spp. during their inaugural year of life, accompanied by an annual incidence rate of 1.2 episodes (Omara et al., 2015; Sainato et al., 2018). Similarly, the current research (Table 2) indicated that the highest incidence occurred in the age group 6 months to 5 years (46.67%). In contrast, the incidence rates were recorded at 6.67% and 13.3% in the age ranges of 6-15 and 16-50 years, respectively. Notably, the pathogen could not be detected within the age range of 51-70 years, yielding an overall detection rate of 28%. This finding corroborates the assertions made by Vaishnav et al. (2015), who reported that 50% of Campylobacter isolates were derived from children under 5 years old, with a subsequent prevalence noted among individuals exceeding 10 years of age.

Lower isolation frequencies of Campylobacter spp. from human stool samples in Egypt were reported; Girgis et al. (2014) in Assiut; and Abd El?Tawab et al. (2015), who reported isolation rates of 2.7, 5.3, and 8%, respectively. On the other hand, Awadallah et al. (2014) in Zagazig recorded a higher rate (56.6%).

Isolation rates among the Egyptian governorates varied from 8.5% to 38.09% for C. jejuni derived from occupational workers (Omara et al., 2015; ElSayed, 2016; Abushahba et al., 2018). Conversely, Sarkar et al. (2014) in Bangladesh, and El-Tawab et al. (2015) in Egypt reported lower percentages (9.3, and 11.5%) of Campylobacter.

C. jejuni and C. coli were identified at a similar rate by ElSayed (2016) in Sohag Governorate, while in Assiut Governorate Abushahba et al. (2018) recorded rates of 11.7% and 6.7% for the respective species.

Awadallah et al. (2014) reported similar isolation rates of C. coli from fresh chicken meat samples at a rate of 10.8%.

The data shown in Table 3 and Figures 2, 3, &4 revealed that the percentage of C. jejuni was 75% (21/28) and C. coli was 25% (7/28), 1 sample had mixed strains. These results are on the same line as those by Abdulwahab and Alhindwae (2025) who reported that the percentage of C. jejuni/C. coli was 61.7, and 20.22%, respectively. Additionally, Abd El‐Tawab et al. (2014) reported that the percentages of C. jejuni, and C. coli, infections were 54.4, and 42.1%, respectively. A possible explanation for these results may be the more sensitive nature of C. coli to stress conditions during the slaughtering process of broilers.

In eggshell samples, 2 isolates from eggshells were positive for the 23S rRNA, C. jejuni was identified in all 2/50 (4%) using the Hippurate test and mPCR, while 1 sample had mixed C. jejuni and C. coli contamination (Tables 1,3 and Figure 4). Also, a predominance of C. jejuni (81.9%) compared to C. coli (18.2%) was obtained in Gharbi et al. (2022).

For the environmental samples, the C. jejuni /C. coli ratio (6/3) identified in the current study was 67.67/33.33%. Similarly, the ratios obtained by (Abd El‐Tawab et al., 2015), C. jejuni, and C. coli/C. lari were identified in 76.9, and 23.1% of the human isolates, respectively. A higher Campylobacter spp. detection rate was reported among workers in percentages of 24% in the 18–50 years age groups by Sayed et al. (2023). (C. jejuni 15%, and C. coli 14%) of the workers examined via mPCR. Higher isolation rates of C. jejuni were reported in different studies by Salihu et al. (2012) and Mansouri-najand et al. (2012). A lower result (31.4%) was reported by Henry et al. (2011).

C. jejuni was identified in 4.14% (5/120) and 0.83% (1/120) of the C. coli strains in the examined human samples (Table 4, and Figures 3,4). Similar percentages of C. jejuni (5.8%) were obtained in France by Bessède et al. (2011). A lower rate (1.5%) was reported in India by Rajagunalan et al. (2014). On the other hand, higher percentages of C. coli (2.5, and 1.5%) were obtained by Bessède et al. (2011), and Rajagunalan et al. (2014), respectively.

In children aged 0.5-5 years, C. jejuni/ C. coli was detected at a rate of 3:1 in the stool samples, and 1:1 in those aged 5-15 years. Similar detection rates of C. jejuni were reported by Abd El‐Tawab et al. (2015) who detected C. jejuni at rates of 70.9%. Awadallah et al. (2014) identified 7.4% and 3.7% as C. coli and C. jejuni, respectively. In contrast, C. coli and C. jejuni as 57.5% versus 0%. were obtained by Marinou et al. (2012). Sayed et al. (2023) reported that C. jejuni and C. coli were recovered from workers in 18–50 years age groups at percentages of 12% each.

CONCLUSION

Campylobacteriosis is a pressing global health issue driven by the contamination of broiler meat and food environments. Poultry and poultry meat are important sources of Campylobacter infections mainly Campylobacter jejuni and Campylobacter coli. Efforts to mitigate Campylobacter contamination must address both preharvest and// postharvest stages. Strategies include improving biosecurity, ensuring hygienic processing, and minimizing cross-contamination. Proper monitoring during the processing and handling of poultry meat is critical to eliminate infection risk.

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