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Article

Prevalence, virulence gene profile and antibiogram of *Campylobacter jejuni* from fresh vegetables in Mymensingh, Bangladesh

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Abstract: This study aimed to investigate *Campylobacter jejuni*, a major cause of food-borne bacterial infections worldwide, in fresh vegetables from five upzillas (Mymensingh, Trishal, Bhaluka, Muktagacha, and Fulbaria) in the Mymensingh district between July 2020 and April 2023. Using cultural, biochemical, and molecular techniques, 100 fresh vegetable samples (including tomato, carrot, cucumber, green chili, and coriander) were examined for *C. jejuni*. The isolates were further tested for virulence genes and antimicrobial susceptibility. Out of the 100 samples, 23% were confirmed as *C. jejuni*, by 16S rRNA gene-based polymerase chain reaction and all were found to be virulent with cytolethal distending toxins (*cdtA, cdtB and cdtC* genes). Antibiotic susceptibility testing revealed resistance to amoxicillin (47.83%), tetracycline (43.48%), and streptomycin (39.13%) among the isolates. However, ceftriaxone and ciprofloxacin were effective against 47.83% and 43.48% of the isolates, respectively. Moreover, 52.17% of the isolates were sensitive to erythromycin. Alarmingly, 34.78% of the *C. jejuni* isolates exhibited multidrug resistance (MDR) with eight different antibiotic resistance patterns, including four MDR patterns. These findings highlight the presence of virulent and antibiotic-resistant *C. jejuni* in fresh vegetables, emphasizing the need for monitoring and control to ensure food safety and public health issues.

Keywords: fresh vegetables; *Campylobacter jejuni*; prevalence; virulence characterization; MDR; food safety; MAR

1. Introduction

Vegetables are widely recognized for their nutritional value, providing vitamins, fiber, micronutrients, and minerals essential for human health. Deficiencies in vitamins C and A can lead to various health issues, underscoring the importance of well-balanced diets with a high vegetable intake (Kalia and Gupta, 2006). Salad vegetables like tomatoes, carrots, cucumbers, green chilies, and coriander leaves are commonly consumed raw in traditional salad preparations worldwide.

In today's society, food safety is a major concern, with microorganisms being a significant factor in food adulteration. Among the potential pathogen contaminants, *Campylobacter* spp. is particularly concerning.

Campylobacter is a well-known cause of bacterial infections, responsible for a majority of cases of acute gastro-intestinal infection in humans globally (Allos, 1998). In developed countries, campylobacteriosis poses a serious public health risk and is a prevalent cause of gastroenteritis (Friedman *et al*., 2000). Among *Campylobacter* species, *Campylobacter jejuni* accounts for more than 95% of identified infections (Altekruse *et al*., 1999). The introduction of enteric pathogens, including *Campylobacter,* via fecal contamination from both urban and rural sources can happen at different stages, including field cultivation and food processing (Kumar *et al*., 2001).

Campylobacter species are bacteria that are Gram-negative, non-spore-forming, and curved rod-shaped, measuring approximately 0.2 to 0.5 μm in width and about 0.5 to 5 μm in length (Doyle, 1990). They thrive in an environment with approximately 5% O_2, 10% CO_2, and 85% N_2, which is considered ideal condition for their optimal growth (Forbes *et al*., 1998). *Campylobacter* is commonly found in the digestive tracts of poultry, cattle, and animal-derived food products and is often associated with cases of diarrhea (de Boer *et al*., 2000). According to Zia *et al*. (2003), *Campylobacter* spp. is highly pathogenic, causing severe diarrhea, reactive arthritis, and even Guillain-Barre syndrome. Campylobacteriosis, the infection caused by these bacteria, is a significant public health concern in many developed countries, and monitoring of infections and antibiotic resistance patterns is ongoing (Kabir *et al*., 2011). Thermotolerant *Campylobacter* is recognized as the most common bacterial cause of foodborne illnesses worldwide (Rossler *et al*., 2020).

The increasing microbial resistance is a global concern due to their extensive use in both human and veterinary practices (Hassan *et al*., 2014). Reports of *Campylobacter* species' resistance to antimicrobial agents have been documented worldwide (Isenbarger *et al*., 2002; Rahman *et al*., 2021), with low and middle income countries (LMICs) experiencing a rapid escalation in resistance due to imprudent antibiotic usage (Englen *et al*., 2003). The excessive use of antimicrobial agents in food animal production has led to a rise in antimicrobial-resistant *Campylobacter* species, negatively impacting both human and animal health in terms of food safety and public health grounds (Engberg *et al*., 2004). Antibiotics are commonly employed in veterinary practices for livestock and poultry production as curative, preventative, and growth-promoting agents.

Given the risks associated with *Campylobacter* contamination, strict sanitary measures, including personal hygiene and food safety, should prohibit the consumption of food products contaminated with this organism. Antibiotics may be used to treat human clinical cases of *Campylobacter* spp. infection following sensitivity testing (Karmaker *et al*., 2018). While a few studies have assessed the microbiological contamination of vegetables in Bangladesh (Nipa *et al*., 2011; Rahman and Noor, 2012; Ohiduzzaman *et al*., 2022), there are currently sparse studies on the occurrance of *C. jejuni* in fresh vegetables, its virulence gene profile, or its antibiograms. This study focuses on raw vegetables like cucumber, green chili, coriander, and tomato, commonly consumed in salads, which pose a significant risk for *Campylobacter* infection. The objective of this study is to isolate and identify *C. jejuni* from fresh vegetables obtained from various local markets, characterize its virulence, and assess trends in antibiotic resistance.

2. Materials and Methods

2.1. Ethical approval

The Ethical Committee of the Bangladesh Agricultural University, Mymensingh, Bangladesh approved the study under reference no. AWEEC/BAU/2020 (12).

2.2. Collection and transportation of samples

One hundred (100) vegetable samples, comprising a mix of tomato, carrot, cucumber, green chili, and coriander, were collected and immediately transported to the Bacteriology Laboratory at the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, while maintaining a cool chain system. The samples were promptly processed upon arrival to identify and isolate *C. jejuni*.

2.3. Isolation of *C. jejuni*

Isolation *C. jejuni* was carried out using the filtering technique (0.45 μm filter) as described by Shiramaru *et al*. (2012). Each vegetable sample was washed with water containing 0.1% peptone, and the filtered samples were placed on top of Blood Agar Base No. 2 with Skirrow Supplement. After pouring a portion of the peptone water over the filters, they were left at room temperature for 30 minutes. Subsequently, the filters were removed, and the plates were incubated under microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2) at 37°C for 48 hours. The incubated media were examined for bacterial growth, and grey, flat, irregularly spreading colonies were observed on Skirrow blood agar. Gram's staining and microscopic observation confirmed the presence of Gram-negative curved bacteria. Oxidase and catalase tests were conducted on selected colonies that exhibited a Gram-negative curve in the smears and were catalase and oxidase positive. These selected colonies were sub-

cultured onto Blood Agar Base No. 2 with Skirrow supplement to obtain single and pure colonies, which appeared as grey, flat, and irregularly spreading colonies on the surface of Skirrow blood agar. The resulting pure isolates were used for further research.

2.4. Molecular identification and virulence characterization by PCR

The DNA extraction from cultured bacteria followed the standard boiling method described by Hoshino *et al*. (1998). For this, 3-5 pure single colonies from Blood base agar were mixed with 250 µl of deionized water in an Eppendorf tube. The tubes were then placed in boiling water and boiled for 10 minutes, followed by immediate transfer to ice for 10 minutes to induce cold shock. Afterward, the tubes were centrifuged at 12,000 rpm for 12 min, and approximately 100 µl of the supernatant was collected as the DNA template for the PCR assay aimed at amplifying the targeted genes.

PCR reactions to amplify various target genes in *C. jejuni* isolates were performed using a Thermocycler (2720 Thermal Cycler, Applied Biosystems, USA) with a 25 µl PCR mixture. The oligonucleotide primer sequences and corresponding target genes used for identifying and characterizing the virulence of *C. jejuni* isolates are listed in Table 1, while the thermal profiles applied for PCR amplification of various genes are presented in Table 2. The PCR products, including the 16S rRNA gene and *hipO, cdtA, cdtB, cdtC* genes, were separated on 1.5% and 2% agarose gels (Invitrogen, USA), respectively, and stained with ethidium bromide (0.5 µg/ml) (Sigma-Aldrich, USA). The gels were then visualized using an ultraviolet transilluminator (BDA digital, Biometra GmbH, Germany).

Table 1. List of oligonucleotide primer sequences and analogous target genes for identification and virulence characterization of *C. jejuni* isolates.

Primer	Sequence (5'-3')	Target genes	Amplicon size	References
16S9F	GAGTTTGATCCTGGCTC	*16S* rRNA	1530 bp	Samosornsuk *et al*. (2007)
16S1540R	AAGGAGGTGATCCAGCC			
HIP400F	GAAGAGGGTTTGGGTGGTG	*hip*O	735 bp	Linton *et al*. (1997)
HIP1134R	AGCTAGCTTCGCATAATAACTTG			
Cj-CdtAU2	AGGACTTGAACCTACTTTTC	*cdtA*	631 bp	Asakura *et al*. (2008)
Cj-CdtAR2	AGGTGGAGTAGTTAAAAACC			
Cc-CdtAU1	ATTGCCAAGGCTAAAATCTC		329 bp	
Cc-CdtAR1	GATAAAGTCTCCAAAACTGC			
Cf-CdtAU1	AACGACAAATGTAAGCACTC		489 bp	
Cf-CdtAR1	TATTTATGCAAGTCGTGCGA			
Cj-CdtBU5	ATCTTTTAACCTTGCTTTTGC	*cdtB*	714 bp	Asakura *et al*. (2008)
Cj-CdtBR6	GCAAGCATTAAAATCGCAGC			
Cc-CdtBU5	TTTAATGTATTATTTGCCGC		413 bp	
Cc-CdtBR5	TCATTGCCTATGCGTATG			
Cf-CdtBU6	GGCTTTGCAAAAACCAGAAG		533 bp	
Cf-CdtBR3	CAAGAGTTCCTCTTAAACTC			
Cj-CdtCU1	TTTAGCCTTTGCAACTCCTA	*cdtC*	524 bp	Asakura *et al*. (2008)
Cj-CdtCR2	AAGGGGTAGCAGCTGTTAA			
Cc-CdtCU1	TAGGGATATGCACGCAAAAG		313 bp	
Cc-CdtCR1	GCTTAATACAGTTACGATAG			
Cf-CdtCU2	AAGCATAAGTTTTGCAAACG		397 bp	
Cf-CdtCR2	GTTTGGATTTTCAAATGTTCC			

Table 2. Number of cycles, amplicon size and thermal profile for the amplification of target genes by PCR.

Target genes	Thermal profile					Number of cycles	Amplicon size (bp)
	Initial denaturation	Denaturation	Annealing	Extension	Final extension		
16SrRNA	94°C/5min	94°C/30sec	47°C/30sec	72°C/90sec	72°C/7min	30	1530
*hip*O	94°C/5min	94°C/30sec	55°C/30sec	72°C/45sec	72°C/7min	30	735
cdtA	94°C/5min	94°C/30sec	53°C/30sec	72°C/30sec	72°C/7min	30	631
cdtB	94°C/5min	94°C/30sec	52°C/30sec	72°C/30sec	72°C/7min	30	714
cdtC	94°C/5min	94°C/30sec	53°C/30sec	72°C/45sec	72°C/7min	30	424

2.5. Antimicrobial susceptibility test

To determine the antimicrobial resistance profiles of the *C. jejuni* isolates, the disk diffusion technique described by Luangtongkum *et al*. (2007) was employed on Mueller-Hinton agar plates, following the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2018). Eight antibiotics were used in the susceptibility testing: Amoxicillin (30 µg/disc), Azithromycin (30 µg/disc), Ciprofloxacin (5 µg/disc), Erythromycin (30 µg/disc), Gentamicin (10 µg/disc), Ceftriaxone (10 µg/disc), Streptomycin (10 µg/disc), and Tetracycline (30 µg/disc). The results were categorized as susceptible (S), intermediate (I), or resistant (R) based on the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI, 2018). According to Sweeney *et al*. (2018), an isolate is considered multidrug resistant (MDR) when it shows resistance to three or more different classes of antimicrobial agents.

2.6. Multiple antimicrobial resistance index (MARI)

The formula used by Msolo *et al*. (2020) to calculate the multiple antimicrobial resistance index (MARI) for *C. jejuni* isolates is as follows:

$$\text{MARI}=\frac{a}{b}$$

where "a" is the number of antibiotics to which a particular isolate is found resistant, and "b" is the total number of antibiotics to which each individual isolate was evaluated.

2.7. Data management and statistical analysis

The data were recorded in a Microsoft Excel 2016 spreadsheet (Microsoft Office 2016, Microsoft, Los Angeles, CA, USA) and analyzed using SPSS version 20. Descriptive statistics, including frequency and percentage, were computed for the analysis.

3. Results

3.1. Isolation and identification of *C. jejuni*

Campylobacter spp. displayed distinct characteristics on Skirrow blood agar plates, showing grey, round, convex, smooth, and shiny colonies with regular edges after being incubated at 37°C for 48 h under microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2). In Gram's staining, the organisms appeared as small, curved, Gram-negative cells arranged singly or in pairs, exhibiting a pink color. The purity of the organisms was confirmed and validated using specialized Blood Agar Base No. 2 media. In the catalase and oxidase tests, *Campylobacter* spp. isolates were found to be positive. The hippurate hydrolysis test indicated positive results only for *C. jejuni*, while *C. coli* was determined to be negative (Table 3).

Table 3. Isolation and identification of *C. jejuni* in vegetables based on culture characteristics on selective media, staining, biochemical tests and PCR assays.

Total no. of samples	No. (%) of positive samples on the basis of						
	Skirrow blood agar (grey color spreading colonies)	Gram's staining (Gram negative, pink color, small curved shape arranged as single or pair)	Oxidase Test	Catalase Test	Hippurate hydrolysis test	16S rRNA gene-based PCR	*hip*O gene-based PCR
100	37 (37)	33 (33)	29 (29)	29 (29)	23 (23)	23 (23)	23 (23)

3.2. Prevalence of *C. jejuni* in fresh vegetables in Mymensingh

For this research, 100 fresh vegetable samples, consisting of tomato, carrot, cucumber, green chili, and coriander, were collected from 5 upzillas (Mymensingh, Trishal, Bhaluka, Muktagacha, and Fulbaria) within the Mymensingh district. The isolation of *C. jejuni* was performed using the filtration technique. Out of the 100 samples tested, a total of 23 samples (23%, 23/100, 95% CI: 15.84-32.15%) tested positive for *C. jejuni* (Table 3).

3.3. Molecular detection by PCR

To confirm the presence of the *Campylobacter* genus, a 16S rRNA gene-based PCR was conducted. This PCR test generated a distinct amplification of 1530 bp in 23 different *Campylobacter* isolates (Figure 1). Furthermore, to validate the identity of the isolates as *C. jejun*i, a targeted *hipO* gene-based PCR was

accomplished. The *hipO* gene-based PCR yielded specific amplification of 735 bp in all 23 *Campylobacter* isolates, confirming their identity as *C. jejuni* (Figure 2).

3.4. Virulence characterization of *C. jejuni* by cdt gene-based multiplex PCR assays

To characterize the virulence of *C. jejuni*, multiplex PCR assays targeting the *cdtA*, *cdtB*, and *cdtC* genes were performed. All 23 *C. jejuni* isolates showed specific amplification at 631 bp, 714 bp, and 524 bp for the *cdtA*, *cdtB, and cdtC* genes, respectively, indicating the presence of these virulence genes in all isolates (Table 4). The PCR results are presented in Figures 3, 4, and 5.

Table 4. Overall prevalence of *C. jejuni* isolates in fresh vegetables in Mymensingh district.

Name of isolate	Positive sample (n)/Tested sample (n)	Prevalence (%) with 95% CI	% of virulence genes					
			On the basis of tested sample			On the basis of positive sample		
			cdtA	*cdtB*	*cdtC*	*cdtA*	*cdtB*	*cdtC*
C. jejuni	23/100	23 (15.84-32.15)	23 (23/100)	23 (23/100)	23 (23/100)	100 (23/23)	100 (23/23)	100 (23/23)

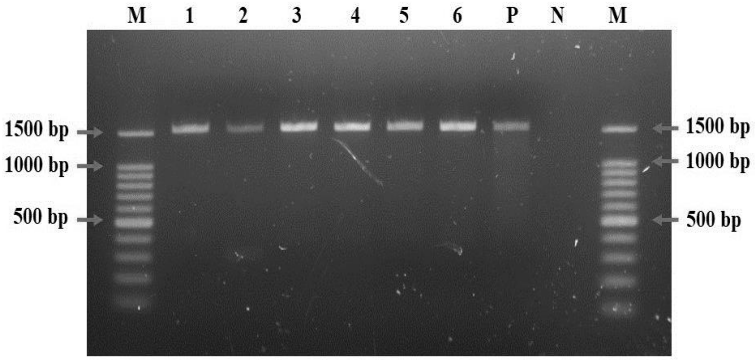

Figure 1. Amplification of 16S rRNA gene (1530 bp). Here, M: 100 bp DNA ladder (Takara, Japan); N: Negative control; P: Positive control (*C. jejuni* ATCC 33560); Lane 1-6: *Campylobacter* isolates positive for 16S rRNA gene.

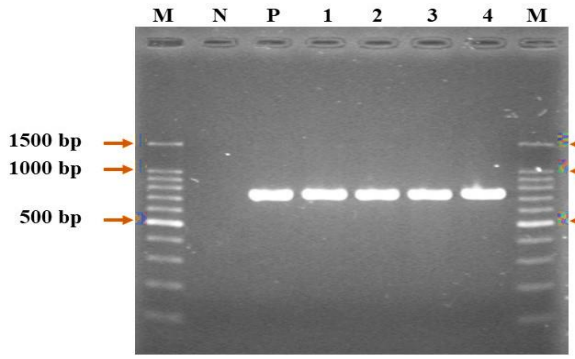

Figure 2. Amplification of *hipO* gene (735 bp). Here, M: 100 bp DNA ladder (Takara, Japan); N: Negative control; P: Positive control (*C. jejuni* ATCC 33560); Lane 1-4: *C. jejuni* isolates positive for *hipO* gene.

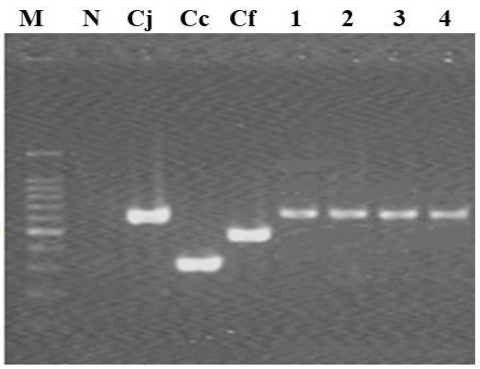

Figure 3. Multiplex PCR assay to amplify *cdtA* gene (631 bp) specific for *C. jejuni*. Here, M: 100 bp DNA ladder (Takara, Japan); N: Negative control; Cj: *C. jejuni* ATCC 33560; Cc: *C. coli* ATCC 33559; Cf: *C. fetus* ATCC 27374; Lane 1-4: *C. jejuni* isolates positive for *cdtA* gene.

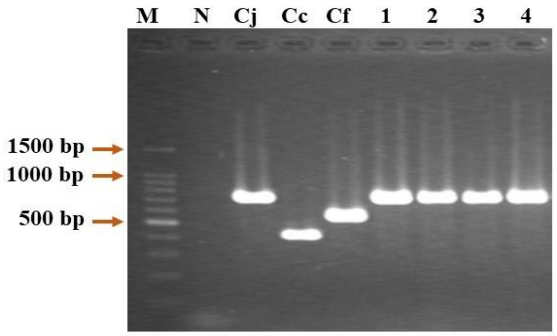

Figure 4. Multiplex PCR assay to amplify *cdtB* gene (714 bp) specific for *C. jejuni.* Here, M: 100 bp DNA ladder (Takara, Japan); N: Negative control; Cj: *C. jejuni* ATCC 33560; Cc: *C. coli* ATCC 33559; Cf: *C. fetus* ATCC 27374; Lane 1-4: *C. jejuni* isolates positive for *cdtB* gene.

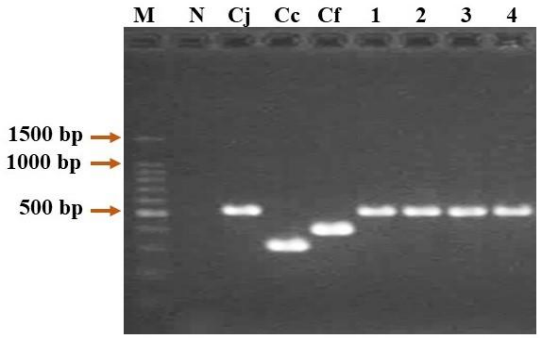

Figure 5. Multiplex PCR assay to amplify *cdtC* gene (524 bp) specific for *C. jejuni.* Here, M: 100 bp DNA ladder (Takara, Japan); N: Negative control; Cj: *C. jejuni* ATCC 33560; Cc: *C. coli* ATCC 33559; Cf: *C. fetus* ATCC 27374; Lane 1-4: *C. jejuni* isolates positive for *cdtC* gene.

3.5. Antimicrobial susceptibility of *C. jejuni* isolated from fresh vegetable

The results of the antimicrobial susceptibility test conducted on the 23 *C. jejuni* isolates using eight commercially available antibiotics from six classes are summarized in Table 5. The antibiogram study revealed that 47.83% (11/23) of the *C. jejuni* isolates were resistant to amoxicillin, and 43.48% (10/23) were resistant to tetracycline. Additionally, 39.13% (9/23) of the *C. jejuni* isolates showed resistance to streptomycin. Surprisingly, ceftriaxone and ciprofloxacin were effective against 47.83% (11/23) and 43.48% (10/23) of the isolates, respectively. Moreover, 52.17% (12/23) of the isolates were found to be sensitive to erythromycin.

Table 5. Antimicrobial susceptibility and resistance patterns of *C. jejuni* isolates against different antibiotics determined by the disk diffusion technique.

Name of isolates	Pattern	Number of isolates (%)							
		AMX	TE	GEN	S	E	AZM	CIP	CTR
C. jejuni (n=23)	S	5 (21.74)	9 (39.13)	11 (47.83)	11 (47.83)	10 (43.48)	12 (52.17)	10 (43.48)	11 (47.83)
	I	7 (30.43)	4 (17.39)	8 (34.78)	9 (39.13)	4 (17.39)	7 (30.43)	5 (21.74)	8 (34.78)
	R	11 (47.83)	10 (43.48)	4 (17.39)	3 (13.04)	9 (39.13)	4 (17.39)	8 (34.78)	4 (17.39)

AMX=Amoxicillin, TE=Tetracycline, GEN=Gentamycin, S=Streptomycin, E=Erythromycin, AZM= Azithromycin, CIP=Ciprofloxacin and CTR=Ceftriaxone

3.6. MDR and MAR profiles of *C. jejuni* isolated from fresh vegetables

The investigation's findings revealed that out of the 23 *C. jejuni* isolates, 34.78% (8/23, 95% CI: 18.81-55.11%) exhibited multidrug-resistant (MDR) phenotypes, with eight different antibiotic resistance patterns, including four MDR patterns. These MDR isolates were identified in 13.4% (3/23) of AMX-S-TE cases, which had the highest MDR pattern. Moreover, two isolates (AMX-TET-ER-GEN) showed resistance to four of the eight antibiotics tested, belonging to six distinct classes. The multiple antibiotic resistance (MAR) indices of the *C. jejuni* isolates ranged from 0.1 to 0.6. The MDR and MAR profiles of the *C. jejuni* isolates from fresh vegetables are presented in Table 6 and Figure 6.

Table 6. Phenotypic MDR profiles of *C. jejuni* isolates from fresh vegetables in Mymensingh district.

No. of pattern	Antibiotic resistance patterns	No. of isolates (%)	No. of antibiotics (class)	No. of MDR isolates (%)	Overall no. of MDR isolates
1	AMX	4 (17.39)	1 (1)	-	-
2	E	3 (13.04)	1 (1)	-	-
3	AMX-TET	5 (21.74)	2 (2)	-	-
4	AMX-S	3 (13.04)	2 (2)	-	-

Table 6. Contd.

No. of pattern	Antibiotic resistance patterns	No. of isolates (%)	No. of antibiotics (class)	No. of MDR isolates (%)	Overall no. of MDR isolates
5	AMX-S-TET	3 (13.04)	3 (3)	3 (13.04)	8 (34.78%, 8/23)
6	E-S-CIP	1 (4.35)	3 (3)	1 (4.35)	
7	AMX-TET-ER	2 (8.70)	3 (3)	2 (8.70)	
8	AMX-TET-ER-GEN	2 (8.70)	4 (4)	2 (8.70)	

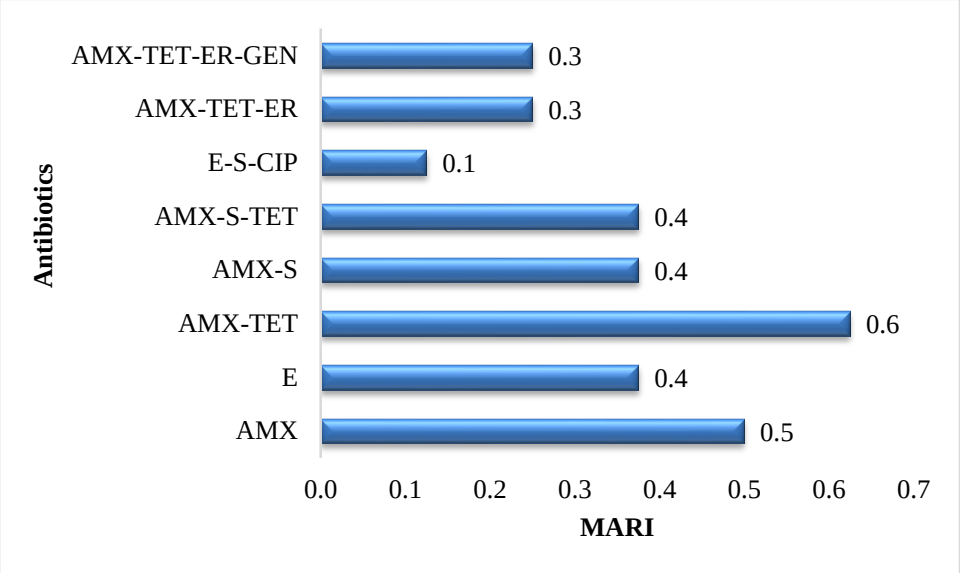

Figure 6. MAR profiles of *C. jejuni* isolates from fresh vegetables in Mymensingh district.

4. Discussion

In this study, *C. jejuni* was isolated and characterized using various techniques, including a cultural features-based study, staining characteristics, biochemical tests, and PCR. To identify *C. jejuni*, the organism was cultured on Blood Agar Base No. 2 with Skirrow supplement, a selective agar media, under microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2). The filtration method (0.45 µm filter paper) was employed to isolate and identify *C. jejuni*, similar to experiments conducted by Haseena (2017) and Forbes *et al*. (1998). The colony characteristics of *C. jejuni* exhibited a pink or light pink color, gram-negative, and slightly curved shape, which were consistent with previous investigations by Kabir *et al*. (2011) and Karmaker *et al*. (2018).

Biochemical tests were conducted for the identification of *C. jejuni*, and the results were in agreement with studies by Shiramaru *et al*. (2012) and Karmaker *et al*. (2018). The oxidase test showed a purple color shift in 23% (23/100) of the isolates, while the hippurate hydrolysis test revealed a strong blue or purple color in all isolates, confirming their identity as *C. jejuni*. The prevalence of *C. jejuni* in this study was found to be 23% (23/100) of the fresh vegetable samples tested. These findings align with previous studies conducted by Rahman *et al*. (2021), who reported a prevalence of 21.8%, and Karmaker *et al*. (2018), who reported a prevalence of 15.33%. The similarity in prevalence rates among these studies indicates a consistent occurrence of *C. jejuni* in fresh vegetables, highlighting the importance of monitoring and ensuring food safety practices to mitigate potential health risks associated with this pathogen.

In this study, the confirmation of the *Campylobacter* genus was achieved using a 16S rRNA gene-based PCR, while the identification of *C. jejuni* was conducted through a targeted hippuricase gene-based (*hipO*) PCR test. The 16S rRNA gene-based PCR resulted in a characteristic amplification of 1530 bp in 23 different *Campylobacter* isolates, consistent with findings from previous studies by Kabir *et al*. (2011) and Rahman *et al*. (2021).

The *hipO* gene-based PCR confirmed the identity of all 23 *Campylobacter* isolates as *C. jejuni*, as they demonstrated specific amplification at 735 bp. Additionally, the virulence of *C. jejuni* was assessed using a multiplex PCR assay targeting the *cdtA, cdtB,* and *cdtC* genes. All 23 *C. jejuni* isolates displayed specific amplification at 631 bp, 714 bp, and 524 bp, respectively, indicating the presence of *cdtA, cdtB,* and *cdtC*

virulence genes. These findings are consistent with other research studies conducted by Kabir *et al*. (2015) and Rahman *et al*. (2021).

The antibiogram study was performed to assess the sensitivity and resistance pattern of the bacterial isolates to various antibiotics commonly used in the market. Among the 23 *C. jejuni* isolates, 47.83% (11/23) were found to be resistant to amoxicillin, and 43.48% (10/23) were resistant to tetracycline. Additionally, 39.13% (9/23) of the isolates exhibited resistance to streptomycin. Surprisingly, ceftriaxone and ciprofloxacin were effective against 47.83% (11/23) and 43.48% (10/23) of the isolates, respectively. Moreover, 52.17% (12/23) of the isolates were sensitive to erythromycin. These findings are similar to those reported by Hakanen *et al*. (2003) and Rahman *et al*. (2021), suggesting a consistent pattern of antibiotic resistance in *C. jejuni* isolates.

Overall, the study utilized a range of techniques to characterize *C. jejuni* isolates, including PCR-based methods for genus and species confirmation, as well as virulence characterization, and an antibiogram study to assess antibiotic resistance patterns. The results provide valuable insights into the prevalence, virulence, and antibiotic resistance of *C. jejuni* in the tested fresh vegetable samples.

The present study revealed a concerning observation of multidrug resistance (MDR) in the majority of *C. jejuni* isolates. Among the 23 *Campylobacter* isolates tested, 34.78% (8/23) were identified as multidrug resistant, defined as being resistant to three or more different antibiotics. This finding aligns with several earlier studies conducted by Hakanen *et al*. (2003), McGill *et al*. (2006), Moore *et al*. (2006), Luangtongkum *et al*. (2009), Karmaker *et al*. (2018), and Rahman *et al*. (2021), which also reported significant proportions of MDR *C. jejuni* isolates.

Furthermore, the study assessed the Multiple Antibiotic Resistance (MAR) index to gain insights into the level of contamination and antibiotic use in the environment from which the isolates were obtained. The MAR index values are an important indicator of the potential risk of antibiotic resistance emergence in the environment. In this study, 21.74% (5/23) of the isolates had MAR indices greater than 0.2, indicating a higher risk of contamination from sources frequently exposed to antibiotics. In contrast, only 4.35% (1/23) of the isolates had MAR indices of 0.2 or less, suggesting lower risk of contamination from antibiotic-exposed sources. This finding is consistent with the observations of Osundiya *et al*. (2013), who associated MAR index values greater than 0.2 with higher-risk sources that are commonly treated with antibiotics.

Additionally, the study compared the MAR indices reported by Beshiru *et al*. (2022) and Fallah *et al*. (2022) with the permissible limit. Both of these studies reported MAR indices exceeding the permissible limit by 0.60 and 0.75 points, respectively. These results further underscore the concern of antibiotic resistance dissemination in various environments, which could have adverse effects on public health and food safety.

5. Conclusions

The escalating bacterial resistance to antibiotics is a worldwide pandemic, posing a significant concern for public health. The extensive and indiscriminate use of antibiotics in food items is a key contributing factor to the development of resistance. The findings of the current study reveal the presence of virulent and multidrug-resistant strains of *C. jejuni* in fresh vegetables within the study area. Considering the potential health risks, isolated cases should be carefully monitored, and risk factors must be minimized by promptly implementing stringent regulations and legislation. To mitigate the risk of *C. jejuni* infections from fresh vegetables, proactive measures should be adopted. Practicing proper hygiene, such as thorough washing of vegetables before cooking and ensuring they are properly cooked, can prove effective in preventing infections. Additionally, the adherence to food safety standards during the processing and handling of fresh vegetables is crucial to reduce the likelihood of contamination. In conclusion, addressing the issue of antibiotic-resistant *C. jejuni* in fresh vegetables necessitates a comprehensive approach involving both regulatory interventions and individual practices.

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Data availability

The data presented in this study are contained in this manuscript.

Conflict of interest
None to declare.

Authors' contribution
Sayed Abdullah-Al-Mamun: Investigation, Methodology, Writing-original draft; M. Rafiqul Islam: Investigation, Methodology, Writing-original draft; Fatema Islam: Investigation; Mohammad Arif: Investigation, Methodology; Yosef Deneke: Writing-review & editing; Sk Shaheenur Islam: Data analysis, Writing-review & editing; Mahmudul Hasan Sikder: Supervision, Writing-review & editing; S. M. Lutful Kabir: Conceptualization, Funding acquisition, Supervision. All authors have read and approved the final manuscript.

References
Allos BM and DN Taylor 1998. *Campylobacter* infections. Bacterial Infections of Humans. Springer, US, pp. 169-190.

Altekruse SF, NJ Stern, PI Fields and DL Swerdlow, 1999. *Campylobacter jejuni* -an emerging foodborne pathogen. Emerg. Infect. Dis., 5: 28.

Asakura M, W Samosornsuk, A Hinenoya, N Misawa, K Nishimura, A Matsuhisa and Yamasaki S, 2008. Development of a cytolethal distending toxin (*cdt*) gene-based species-specific multiplex PCR assay for the detection and identification of *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter fetus*. FEMS Immunol. Med. Microbiol., 52: 260-266.

Beshiru A, AI Okoh and EO Igbinosa, 2022. Processed ready-to-eat (RTE) foods sold in Yenagoa Nigeria were colonized by diarrheagenic *Escherichia coli* which constitute a probable hazard to human health. PLOS ONE, 17: e0266059.

CLSI, 2018. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 22nd informational supplement. CLSI Document M-100 S22.Wayne, Pennsylvania. Clinical and Laboratory Standards Institute. Vol. 32 No. 3.

de Boer P, B Duim, A Rigter, J van der Plas, WF Jacobs-Reitsma and JA Wagenaar, 2000. Computer-assisted analysis and epidemiological value of genotyping methods for *Campylobacter jejuni* and *Campylobacter coli.* J. Clin. Microbiol., 38: 1940-1946.

Doyle MP 1990: *Campylobacter jejuni.* In D. O. Cliver (ed.), Foodborne diseases. Academic Press, Inc., Boston, MA. pp. 217-222.

Engberg J, J Neimann, EM Nielsen, FM Aarestrup and V Fussing, 2004. Quinolone-resistant *Campylobacter* infections: risk factors and clinical consequences. Emerg. Infect. Dis., 10: 1056-1063.

Englen MD, SR Ladely and PJ Fedorka-Cray, 2003. Isolation of *Campylobacter* and identification by PCR. Methods Mol. Biol., 216: 109-121.

Fallah N, M Ghaemi, K Ghazvini, M Rad and A Jamshidi, 2021. Occurrence, pathotypes, and antimicrobial resistance profiles of diarrheagenic *Escherichia coli* strains in animal source food products from public markets in Mashhad, Iran. Food Control, 121: 107640.

Forbes KC, C Pitarangsi, N Vithayasai and L Bodhidatta, 1998. Comparative antibiotic resistance of diarrheal pathogens from Vietnam and Thailand. Emerg. Infect. Dis., 8: 175-180.

Friedman CJ, J Neiman, HC Wegener and RV Tauxe, 2000. Epidemiology of *Campylobacter jejuni* infection in the United States and other industrialized nations. American Soc. Microbiol. Press., pp.121-138.

Hakanen AJ, M Lehtopolku, A Siitonen, P Huovinen and P Kotilainen 2003. Multidrug resistance in *Campylobacter jejuni* strains collected from Finnish patients during 1995–2000. J. Antimicrob. Chemother., 52: 1035-1039.

Haseena M, MF Malik, A Javed, S Arshad, N Asif, S Zulfiqar and J Hanif, 2017. Water pollution and human health. Environmental Risk Assessment and Remediation, 1: 16-19.

Hassan MM, KB Amin, M Ahaduzzaman, M Alam, MS Faruk and I Uddin, 2014. Antimicrobial resistance pattern against *E. coli* and *Salmonella* in layer poultry. Research Journal for Veterinary Practitioners, 2: 30-35.

Hoshino K, S Yamasaki, AK Mukhopadhyay, S Chakraborty, A Basu, SK Bhattacharya, GB Nair, T Shimada and Y Takeda, 1998. Development and evaluation of a multiplex PCR assay for rapid detection of toxigenic *Vibrio cholerae* O1 and O139. FEMS Immunol. Med. Microbiol., 20: 201-207.

Isenbarger DW, CW Hoge, A Srijan, C Pitarangsi, N Vithayasai, LBodhidatta, KW Hickey and PD Cam, 2002. Comparative antibiotic resistance of diarrheal pathogens from Vietnam and Thailand, 1996-1999. Emerg. Infect. Dis., 8: 175-180.

Kabir SML, 2011. Comparison of molecular methods for the species identification of clinical *Campylobacter* strains and their antimicrobial resistance. PhD thesis, submitted to the Department of Veterinary Science, Graduate School of Life and Environmental Sciences, OPU, Osaka, Japan. pp. 1-158.

Kabir SML, K Kikuchi, M Asakura, S Shiramaru, N Tsuruoka, A Goto, A Hinenoya and S Yamasaki, 2011. Evaluation of a cytolethal distending toxin (*cdt*) gene-based species-specific multiplex PCR assay for the identification of *Campylobacter* strains isolated from diarrheal patients in Japan. Japanese J. Infect. Dis., 64: 19-27.

Kabir SML, M Asakura, S Shiramaru, A Pal, A Hinenoya and S Yamasaki, 2015. Molecular identification and antimicrobial resistance profiles of *Campylobacter* strains of poultry origin in India with special emphasis on fluoroquinolone resistance. Asian J. Biol. Res., 1: 1-8.

Kabir SML, N Chowdhury, M Asakura, S Shiramaru, K Kikuchi, A Hinenoya, SB Neogi and S Yamasaki, 2019. Comparison of established PCR assays for accurate identification of *Campylobacter jejuni* and *Campylobacter coli.* Japanese J. Infect. Dis., 72: 81–87.

Kalia A and RP Gupta, 2006. Fruit microbiology. Handbook of fruits and fruit processing, 5: 3-28.

Karmaker S, SML Kabir, AKMZ Haque, MFR Khan and YA Sarker, 2018. Screening of human diarrhoeal samples in Mymensingh city of Bangladesh for the isolation, identification and antimicrobial resistance profiles of *Campylobacter* spp. African. J. Microbiol. Res., 12: 771-778.

Kumar A, RK Agarwal, KN Bhilegaonkar, BR Shome and VN Bachhil, 2001. Occurrence of *Campylobacter jejuni* in vegetables. Int. J. Food Microbiol., 67: 153-155.

Linton D, AJ Lawson, RJ Owen and JP Stanley, 1997. PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. J. Clin. Microbiol., 35: 2568-2572.

Luangtongkum T, B Jeon, J Han, P Plummer, CM Logue and Q Zhang, 2009. Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. Future Microbiol., 4: 189-200.

McGill K, D Cowley, L Moran, P Scates, A O'Leary, RH Madden, C Carroll, E McNamara, JE Moore, S Fanning, JD Collins and P Whyte, 2006. Antibiotic resistance of retail food and human *Campylobacter* isolates on the island of Ireland from 2001-2002. Epidemiol. Infect., 134: 1282-1291.

Moore JE, MD Barton, IS Blair, D Corcoran, JS Dooley, S Fanning, I Kempf, AJ Lastovica, CJ Lowery, M Matsuda, DA McDowell, A McMahon, BC Millar, JR Rao, PJ Rooney, BS Seal, WJ Snelling and O Tolba, 2006.The epidemiology of antibiotic resistance in *Campylobacter*. Microbes and Infection, 8: 1955-1966.

Msolo L, B C Iweriebor and A I Okoh, 2020. Antimicrobial resistance profiles of diarrheagenic *E. coli* (DEC) and *Salmonella* species recovered from diarrheal patients in selected rural communities of the Amathole District Municipality, Eastern Cape Province, South Africa. Infect. Drug Resist., 13: 4615–4626.

Nipa MN, HIM Reaz, MM Hasan, M Fakruddin, H Islam and R Habibur 2011. Prevalence of multidrug resistant bacteria on raw salad vegetables sold in major markets of Chittagong City, Bangladesh. Middle East J. Sci. Res., 10: 70-77.

Ohiduzzaman, SMA Islam, M Arif, MJI Saddam, J Uddain, MT Hossain and SML Kabir, 2022. Microbial quality evaluation of fresh vegetables from distinct markets in urban areas of Bangladesh. Asian Australas. J. Food Saf. Secur., 6: 1-9.

Osundiya O, R Oladele and O Oduyebo, 2013. Multiple Antibiotic Resistance (MAR) indices of *Pseudomonas* and *Klebsiella* species isolates in Lagos University Teaching Hospital. African J. Clin. Exp. Microbiol., 14: 164-168.

Rahman F and R Noor, 2012. Prevalence of pathogenic bacteria in common salad vegetables of Dhaka metropolis. Bangladesh J. Bot., 41: 159-162.

Rahman M, PR Paul, N Hoque, SS Islam, AK Haque, MH Sikder, A Matin, S Yamasaki and SML Kabir, 2021. Prevalence and antimicrobial resistance of *Campylobacter* species in diarrheal patients in Mymensingh, Bangladesh. BioMed Res. Int., 2021: 9229485.

Rossler E, C Olivero, PS Lorena, SF Laureano, J Zimmermann, RR Marcelo, JS Gabriel, LS Marcelo and ZM Virginia, 2020. Prevalence, genotypic diversity and detection of virulence genes in thermotolerant *Campylobacter* at different stages of the poultry meat supply chain. Int. J. Food Microbiol., 326: 108641.

Samosornsuk W, M Asakura, E Yoshida, T Taguchi, K Nishimura, B Eampokalap, V Phongsisay, W Chaicumpa and S Yamasaki, 2007. Evaluation of a cytolethal distending toxin (*cdt*) gene-based species-specific multiplex PCR assay for the identification of *Campylobacter* strains isolated from poultry in Thailand. Microbiol. Immunol., 51: 909-917.

Shiramaru S, M Asakura, H Inoue, A Nagita, A Matsuhisa and S Yamasaki, 2012. A cytolethal distending toxin gene-based multiplex PCR assay for detection of *Campylobacter* spp. in stool specimens and comparison with culture method. J. Vet. Med. Sci., 74: 857-862.

Sweeney MT, BV Lubbers, S Schwarz & JL Watts, 2018. Applying definitions for multidrug resistance, extensive drug resistance and pandrug resistance to clinically significant livestock and companion animal bacterial pathogens. J. Antimicrob. Chemother., *73*: 1460–1463.

Zia S, D Wareing, C Sutton, E Bolton, D Mitchell and JA Goodacre, 2003. Health problems following *Campylobacter jejuni* enteritis in a Lancashire population. Rheumatology 42: 1083-1088.