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% O₂, 10% CO₂, and 85% N₂, 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-Barré 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 occurrence 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% O₂, 10% CO₂, and 85% N₂) 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.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’-3’)</th>
<th>Target genes</th>
<th>Amplicon size</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S9F</td>
<td>GAGTTTGATCCTGGCTC</td>
<td>16S rRNA</td>
<td>1530 bp</td>
<td>Samosornsuk et al. (2007)</td>
</tr>
<tr>
<td>16S1540R</td>
<td>AAGGAGGATGCACAGCC</td>
<td><em>hipO</em></td>
<td>735 bp</td>
<td>Linton et al. (1997)</td>
</tr>
<tr>
<td>HIP400F</td>
<td>GAAGAGGGTTGGGTGTG</td>
<td><em>cdtA</em></td>
<td>631 bp</td>
<td>Asakura et al. (2008)</td>
</tr>
<tr>
<td>HIP1134R</td>
<td>AGCTAGCTTCGATATAACCTT</td>
<td><em>cdtB</em></td>
<td>413 bp</td>
<td>Asakura et al. (2008)</td>
</tr>
<tr>
<td>Cj-CdtA1U</td>
<td>GAGGACTTGAACCTATCTTC</td>
<td><em>cdtC</em></td>
<td>397 bp</td>
<td>Asakura et al. (2008)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Target genes</th>
<th>Initial denaturation</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>Final extension</th>
<th>Number of cycles</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16SrRNA</td>
<td>94°C/5min</td>
<td>94°C/30sec</td>
<td>47°C/30sec</td>
<td>72°C/90sec</td>
<td>72°C/7min</td>
<td>30</td>
<td>1530</td>
</tr>
<tr>
<td><em>hipO</em></td>
<td>94°C/5min</td>
<td>94°C/30sec</td>
<td>55°C/30sec</td>
<td>72°C/45sec</td>
<td>72°C/7min</td>
<td>30</td>
<td>735</td>
</tr>
<tr>
<td><em>cdtA</em></td>
<td>94°C/5min</td>
<td>94°C/30sec</td>
<td>53°C/30sec</td>
<td>72°C/30sec</td>
<td>72°C/7min</td>
<td>30</td>
<td>631</td>
</tr>
<tr>
<td><em>cdtB</em></td>
<td>94°C/5min</td>
<td>94°C/30sec</td>
<td>52°C/30sec</td>
<td>72°C/30sec</td>
<td>72°C/7min</td>
<td>30</td>
<td>714</td>
</tr>
<tr>
<td><em>cdtC</em></td>
<td>94°C/5min</td>
<td>94°C/30sec</td>
<td>53°C/30sec</td>
<td>72°C/45sec</td>
<td>72°C/7min</td>
<td>30</td>
<td>424</td>
</tr>
</tbody>
</table>
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% O₂, 10% CO₂, and 85% N₂). 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.

<table>
<thead>
<tr>
<th>Total no. of samples</th>
<th>16S rRNA gene-based PCR</th>
<th>hipO gene-based PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>23 (23)</td>
<td>23 (23)</td>
</tr>
<tr>
<td>Skirrow blood agar (grey color spreading colonies)</td>
<td>Oxidase Test</td>
<td>Catalase Test</td>
</tr>
<tr>
<td>Gram's staining (Gram negative, pink color, small curved shape arranged as single or pair)</td>
<td>29 (29)</td>
<td>29 (29)</td>
</tr>
</tbody>
</table>

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. jejuni*, 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.

<table>
<thead>
<tr>
<th>Name of isolate</th>
<th>Positive sample (n)/Tested sample (n)</th>
<th>Prevalence (%) with 95% CI</th>
<th>% of virulence genes On the basis of tested sample</th>
<th>% of virulence genes On the basis of positive sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. jejuni</td>
<td>23/100</td>
<td>23 (15.84-32.15)</td>
<td>23 (23/100)</td>
<td>23 (23/100)</td>
</tr>
</tbody>
</table>

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.
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.

<table>
<thead>
<tr>
<th>Name of isolates</th>
<th>Pattern</th>
<th>AMX</th>
<th>TE</th>
<th>GEN</th>
<th>S</th>
<th>E</th>
<th>AZM</th>
<th>CIP</th>
<th>CTR</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. jejuni</em> (n=23)</td>
<td>S</td>
<td>5 (21.74)</td>
<td>9 (39.13)</td>
<td>11 (47.83)</td>
<td>11 (47.83)</td>
<td>10 (43.48)</td>
<td>12 (52.17)</td>
<td>10 (43.48)</td>
<td>11 (47.83)</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>7 (30.43)</td>
<td>4 (17.39)</td>
<td>8 (34.78)</td>
<td>9 (39.13)</td>
<td>4 (17.39)</td>
<td>7 (30.43)</td>
<td>5 (21.74)</td>
<td>8 (34.78)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>11 (47.83)</td>
<td>10 (43.48)</td>
<td>4 (17.39)</td>
<td>3 (13.04)</td>
<td>9 (39.13)</td>
<td>4 (17.39)</td>
<td>8 (34.78)</td>
<td>4 (17.39)</td>
</tr>
</tbody>
</table>

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) 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.

<table>
<thead>
<tr>
<th>No. of pattern</th>
<th>Antibiotic resistance patterns</th>
<th>No. of isolates (%)</th>
<th>No. of antibiotics (class)</th>
<th>No. of MDR isolates (%)</th>
<th>Overall no. of MDR isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AMX</td>
<td>4 (17.39)</td>
<td>1 (1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>E</td>
<td>3 (13.04)</td>
<td>1 (1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>AMX-TET</td>
<td>5 (21.74)</td>
<td>2 (2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>AMX-S</td>
<td>3 (13.04)</td>
<td>2 (2)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
### Table 6. Contd.

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

**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% O₂, 10% CO₂, and 85% N₂). 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* genes.
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.

### Acknowledgements

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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.

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