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Prevalence, Antibiogram, and *cdt* Genes of Toxigenic *Campylobacter jejuni* in Salad Style Vegetables (Ulam) at Farms and Retail Outlets in Terengganu

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ABSTRACT

The present study was conducted to investigate the prevalence and antibiotic resistance among *Campylobacter jejuni* in ulam at farms and retail outlets located in Kuala Terengganu, Malaysia. A total of 526 samples (ulam, soil, and fertilizer) were investigated for the presence of *C. jejuni* and the gene for cytolethal distending toxin (*cdt*) by using a multiplex PCR method. Antibiotic susceptibility to 10 types of antibiotics was determined using the disk diffusion method for 33 *C. jejuni* isolates. The average prevalence of contaminated samples from farms, wet markets, and supermarkets was 35.29, 52.66, and 69.88%, respectively. The *cdt* gene was not detected in 24 of the 33 *C. jejuni* isolates, but 9 isolates harbored *cdtC*. Antibiotic resistance in *C. jejuni* isolates was highest to penicillin G (96.97% of isolates) followed by vancomycin (87.88%), ampicillin (75.76%), erythromycin (60.61%), tetracycline (9.09%), amikacin (6.06%), and norfloxacin (3.03%); none of the isolates were resistant to ciprofloxacin, enrofloxacin, and gentamicin. In this study, *C. jejuni* was present in ulam, and some isolates were highly resistant to some antibiotics but not to quinolones. Thus, appropriate attention and measures are required to prevent *C. jejuni* contamination on farms and at retail outlets.

Ulam is a local name for malay traditional vegetables consisting of plants or parts of plants from 120 species, representing many families from herbs to trees *(34)*, that can be eaten raw or minimally cooked. Ulam makes up an important part of the food intake among the local people, especially the Malay and indigenous communities, and is very popular among all Malaysians and among tourists who have acquired a taste for it. Traditionally, ulam is served raw and flavored with fish or shrimp sauce. Intake of leafy green vegetables is projected to continue to grow *(10)* because of their nutritional benefits *(50)*. According to Tey et al. *(55)*, per capita consumption of vegetables in Malaysia increased from 7.25 kg in 1982 to 40.58 kg in 2001.

An increase in the consumption of ready-to-eat or minimally cooked vegetables increases the risk of foodborne illness because pathogens can be part of the indigenous microflora of vegetables *(22)*. Fresh produce, which was once promoted as a healthy food, can now be a vehicle foodborne illnesses *(6, 13, 38, 39, 48, 51)*. Foodborne illness outbreaks due to fresh produce are increasing *(8, 11, 14)* and have been caused by microorganisms such as bacteria, viruses, and parasites *(9, 17)*. From 2002 to 2011, 684 produce-related outbreaks occurred in the United States *(18)*.

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Surveillance of vegetables has indicated that these minimally processed foods can be contaminated with various bacterial pathogens, including *Staphylococcus, Shigella, Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Campylobacter jejuni (13, 20, 27)*. *Campylobacter* has been recognized as a major cause of human bacterial gastroenteritis, causing millions of cases worldwide every year *(24)*. Toxin production by *C. jejuni* has been identified as one of the potential virulence factors *(21)*. Cytolethal distending toxin (CDT) is produced by the *cdt* gene, which comprises *cdtA, cdtB*, and *cdtC (3, 35, 40, 43)*. CDT affects the epithelial cell layer, resulting in progressive distension and death of the cell *(30, 40)*. *Campylobacter* strains with a *cdt* gene have been isolated from humans and other animals *(4, 5, 43)*. Recently, *Campylobacter* has been found in fresh produce *(7, 16)*, thus posing a risk of campylobacteriosis from the consumption of contaminated fresh produce.

Limited studies and few data are available in Malaysia on the occurrence of *Campylobacter* in food ranging from fresh produce to animal sources. Chai et al. *(16)* reported a high prevalence of *Campylobacter* in vegetables in Selangor, Malaysia, highlighting the importance of monitoring this pathogen in fresh produce, especially ready-to-eat vegetables. As few as 500 to 800 cells of a highly virulent *Campylobacter* strain can cause foodborne illness

TABLE 1. *Type and number of raw vegetable samples*

Scientific name	Local name	English name	*n*
Psophocarpus tetragonolobus	Kacang botol	Winged bean	61
Vigna unguiculata	Kacang panjang	Long yard bean	60
Cantella asiatica	Pegaga	Indian pennywort	20
Ipomoea aquatica	Kangkung	Water spinach	55
Cucumis sativus	Mentimun	Cucumber	23
Brassica oleracea	Kobis	Cabbage	30
Cosmos caudatus	Ulam raja	Wild cosmos	70
Oenanthe javanica	Selom	Japanese parsley	47
Poligonum minus	Kesum	Vietnamese coriander	10
V. radiata	Tauge	Mung bean sprout	10

in humans *(12, 41)*. The goal of this study was to determine the presence of toxigenic *C. jejuni* in ulam at farms and retail outlets. Antibiotic resistance among *C. jejuni* isolates from ulam also was determined.

MATERIALS AND METHODS

Sampling technique. A total of 526 samples of vegetables (386 samples), soils (80 samples), and fertilizers (60 samples) were collected from farms and retail outlets in Terengganu, Malaysia. Five farms (Pulau Kerengga, Bukit Kor, Kampung Alur Parit, Kampung Durian Mas, and Maras), two wet markets (Wakaf Mempelam and Syahbandar), and two supermarkets in Kuala Terengganu were selected as sampling locations. Each of the farms produced various types of crops throughout the year depending on the weather condition.

Vegetables, soil, and fertilizer samples were collected from five farms. The types of raw vegetables selected in this study are listed in Table 1, and the characteristics of each farm are listed in Table 2. Farmers applied fertilizer at least one or two times every month once they started to plant the vegetables. Most farmers use manure in addition to types of chemical fertilizer. Crops were harvested periodically depending on the type of crop.

Detection of *C. jejuni*. Vegetable samples were enriched with Bolton selective enrichment broth (BB) without blood as described by Williams et al. *(57)*. Ten grams of sample was mixed with 90 ml of BB (CM0983B, Oxoid, Basingstoke, England) supplemented with modified Bolton broth selective supplement (SR0208E, Oxoid) and homogenized for 60 s with a stomacher (Seward, Worthington, UK). The enriched samples were incubated at 42°C for 48 h in an anaerobic jar (0.4-liter AnaeroPack rectangular jar; Fisher Scientific, Waltham, MA) under microaerophilic conditions with Anaerocult C (Merck, Darmstadt, Germany).

DNA was extracted from the enriched samples with the boiled-cell method with minor modifications as described by Tang et al. *(52, 53)*. One milliliter of enriched broth was centrifuged at 12,000 × *g* for 5 min to pellet the bacterial cells. The pellet was resuspended in 300 μl of Tris-EDTA buffer and boiled for 10 min in a Corning LSE digital dry bath heater (Sigma Aldrich, Gillingham, UK). Boiled samples were cooled at −20°C for 10 min and then centrifuged at 12,000 × *g* for 5 min. The supernatant (100 μl) was transferred to a new microcentrifuge tube and kept at −20°C until further processing by PCR.

A multiplex PCR method was used to detect *C. jejuni* and *cdt* genes. Specific primers used in this study are listed in the Table 3. All the oligonucleotide primers were synthesized by First BASE Laboratories Sdn. Bhd. (Selangor, Malaysia). DNA extracted from *C. jejuni* ATCC 33560 (Microbiologics, St. Cloud, MN) was used as a control. The multiplex PCR was performed in 25 μl of reaction mixture with a final concentration of 1× Green GoTaq Flexi buffer, 0.2 mM concentration of the deoxynucleotide triphosphate mix, 0.2 μM concentrations of each primer, 3 mM $MgCl_2$ solution, 2 U of GoTaq DNA polymerase, and 2 μl of DNA boiled lysate. All items used in the PCR were purchased from Promega (Madison, WI). PCRs were performed on a Veriti 96-well Fast Thermal Cycler (Applied Biosystems, Foster City, CA), with an initial denaturation step of 95°C for 5 min followed by 30 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 30 s, and a final extension step at 72°C for 5 min.

PCR products were visualized by electrophoresis in a 1.5% agarose gel at 70 V for 90 min. Bands were visualized with UV light after staining with GelRed nucleic acid gel stain (Biotium, Hayward, CA). A 100-bp DNA ladder (NL1405, Vivantis, Oceanside, CA) was used as a DNA molecular ladder.

C. jejuni was isolated as described by Tang et al. *(54)* using modified charcoal cefoperazone deoxycholate agar (mCCDA; CM0739B, Oxoid) supplemented with CCDA selective supplement (SR0155, Oxoid). A 0.1-ml portion of enrichment culture was plated in duplicate and incubated at 42°C for 48 h under microaerophilic conditions. Colonies on the plates with a morphology consistent with *Campylobacter* were further confirmed by the colony PCR method. Confirmed *C. jejuni* isolates were stored in glycerol at −20°C.

TABLE 2. *Characteristics of the vegetable farms*

Characteristic	Farm I	Farm II	Farm III	Farm IV	Farm V
Location	Flat land, village	Hill, forest	Flat land, village	Paddy field, village	Flat land, village
Management	Traditional	Traditional	Traditional	Traditional	Traditional
Fertilizer	Chemical fertilizer, treated manure	Chemical fertilizer, treated manure	Chemical fertilizer, treated manure	Chemical fertilizer, own processed manure	Chemical fertilizer, treated manure
Fencing	Yes	Yes	Yes	No	Yes
Environmental factors	Wild birds	Wild birds	Wild birds	Wild birds, cows, buffalo, ducks, other poultry	Wild birds

TABLE 3. *Primers and amplicon size for* Campylobacter jejuni *and* cdt

Primer no.	Target gene	PCR sequence (5′ to 3′)	Amplicon size (bp)	Reference
1	23S rRNA	F: TATACCGGTAAGGAGTGCTGGAG R: ATCAATTAACCTTCGAGCACCG	650	Wang et al. *(56)*
2	*cdtA*	F: CTAT-TACTCCTATTACCCCACC R: AATTTGAACCGCTGTATTGCTC	422	Martinez et al. *(35)*
3	*cdtB*	F: AGGAACTTTACCAAGAA-CAGCC R: GGTGGAGTATAGG-TTTGTTGTC	531	Martinez et al. *(35)*
4	*cdtC*	F: ACTCCTACTGGA-GATTTGAAAG R: CACAGCTGA-AGTTGTTGTTGGC	339	Martinez et al. *(35)*

Antimicrobial susceptibility testing. A total of 33 strains of *C. jejuni* were isolated from raw salad vegetables and soil samples from farms and retail outlets in Terengganu: 9 isolates from supermarkets, 9 isolates from farms, and 15 isolates from wet markets. Antibiotic resistance patterns were determined with the disk diffusion method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) *(37)*. All isolates were grown in BB with supplement (Oxoid) and without lysed horse blood for 48 h at 42°C. A sterile cotton swab was used to uniformly spread *C. jejuni* from broth into Mueller-Hinton agar (Merck). The tested antibiotics were penicillin G (10 μg), tetracycline (30 μg), ciprofloxacin (5 μg), enrofloxacin (5 μg), erythromycin (15 μg), gentamicin (120 μg), norfloxacin (10 μg), amikacin (30 μg), vancomycin (5 μg), and ampicillin (10 μg). Inoculated plates with antibiotic disks were incubated at 42°C for 48 h under microaerophilic conditions. After incubation, the diameter of the inhibition zones was recorded, and the level of susceptibility was determined according to NCCLS guidelines.

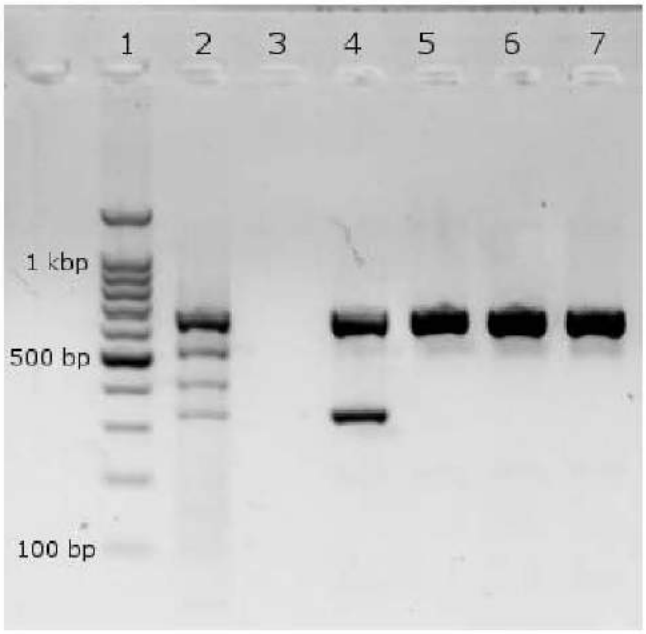

FIGURE 1. *Multiplex PCR detection and identification of the* cdtA *(422 bp),* cdtB *(531 bp),* cdtC *(339 bp), and 23S rRNA (650 bp) genes of* C. jejuni. *Lane 1, 100-bp DNA ladder (Vivantis); lane 2,* C. jejuni *ATCC 33560 reference strain; lane 3, negative control; lane 4, sample positive for 23S rRNA (650 bp) and* cdtC *(339 bp); lanes 5 through 7, samples positive for 23S rRNA (650 bp).*

RESULTS

A total of 526 samples were examined for the prevalence of *Campylobacter* spp. and the presence of *cdt* genes using a multiplex PCR method. Figure 1 shows a representative electrophoresis gel image of the PCR amplification of the 23S rRNA gene and the *cdt* genes.

The prevalence of *C. jejuni* isolates and the presence of *cdt* are summarized in Tables 4 through 6. *C. jejuni* was found in raw vegetables in supermarkets: 66.04 and 76.67% of samples from supermarket I ($n = 53$) and supermarket II ($n = 30$), respectively. Average prevalence of *C. jejuni* at wet markets was 49.20% at wet market I and 60.00% at wet market II. For farm samples, *C. jejuni* occurrence ranged from 15.38 to 60.00% of samples. Cabbage and water spinach had the highest prevalence of *C. jejuni* at 90.00% each, and *cdtC* prevalence ranged from 10.00 to 80.00% with no detection of *cdtA* or *cdtB*. *C. jejuni* was found in all long yard bean samples from market II, and 70.00% of these isolates harbored *cdtC*.

Samples from five farms revealed a *C. jejuni* prevalence of 6.00 to 90.00% in the raw vegetables, 10.00 to 40.00% in fertilizer, and 15.00 to 70% in soil. No *cdtA* or *cdtB* genes were detected in any sample at the farms, and these samples had a low average prevalence of *cdtC* (2.00 to 25.00%).

All 33 isolates of *C. jejuni* were tested against 10 types of antibiotics frequently used in clinical and agricultural settings (Table 7). Most *C. jejuni* isolates (95.70%) were resistant to penicillin G, followed by vancomycin (82.6% of isolates), ampicillin (65.20%), and erythromycin (43.50%). All *C. jejuni* isolates were susceptible to ciprofloxacin, enrofloxacin, and gentamicin. *C. jejuni* resistance to other antibiotics used in this study was low: 4.30% for norfloxacin and 8.70% for both of amikacin and tetracycline.

DISCUSSION

In their study, Chai et al. *(16)* detected *C. jejuni* at every location. Those results indicated that raw salad vegetables in Malaysia were contaminated with *Campylobacter* and pose health risks to consumers. In the present study, *C. jejuni* was detected most frequently at supermarkets. This finding is in close agreement with those of Chai et al. *(16)*, who reported that vegetables at supermarkets were at high risk of becoming contaminated with *C. jejuni*. Such contamination might due to cross-contamination at the supermarket or improper food chain management between the farm and the

TABLE 4. *Supermarket samples that were positive for* C. jejuni *and isolates that were positive for* cdt

			No. (%) of samples							
			PCR				mCCDA			
Location	Vegetable type[a]	No. of samples	*C. jejuni*	*cdtA*	*cdtB*	*cdtC*	*C. jejuni*	*cdtA*	*cdtB*	*cdtC*
Supermarket I	A	10	5 (50.00)	ND[b]	ND	6 (60.00)	ND	ND	ND	ND
	B	10	7 (70.00)	ND	ND	1 (10.00)	5 (50.00)	ND	ND	ND
	C	10	6 (60.00)	ND	ND	1 (10.00)	ND	ND	ND	ND
	D	3	ND	ND	ND	ND	ND	ND	ND	ND
	F	10	9 (90.00)	ND	ND	2 (20.00)	4 (40.00)	ND	ND	1 (10.00)
	G	10	8 (80.00)	ND	ND	8 (80.00)	ND	ND	ND	ND
Total		53	35 (66.04)	ND	ND	18 (33.96)	9 (16.98)	ND	ND	1 (1.89)
Supermarket II	A	10	8 (80.00)	ND	ND	ND	ND	ND	ND	ND
	B	10	6 (60.00)	ND	ND	7 (70.00)	ND	ND	ND	ND
	D	10	9 (90.00)	ND	ND	ND	ND	ND	ND	ND
Total		30	23 (76.67)	ND	ND	7 (23.33)	ND	ND	ND	ND

[a] A, winged bean; B, long yard bean; C, Indian pennywort; D, water spinach; F, cabbage; G, mung bean sprout.
[b] ND, not detected.

retail outlet. The arrangement of the vegetable display area in the supermarket was next to the wet section, which sells seafood, chicken, and meat; this proximity might have contributed to the cross-contamination. *C. jejuni* is highly prevalent in retail poultry meat in Malaysia *(45, 50, 52, 54)*, which increases the likelihood that *Campylobacter* could transfer from the wet section to the fresh produce section via retailers, handlers, or customers *(36)*.

The prevalence of *C. jejuni* in the samples from farm IV (60.00%) was the highest among all the investigated farms (Table 6). Several factors might have contributed to this high prevalence. Contamination may have come from the wild birds and livestock, such as cows and buffalo, due to the lack of proper fencing, whereas farms I, II, III, and V were fenced to prevent livestock from damaging their plants (Table 2). Wild birds and livestock can contaminate vegetables through fecal material that may contain *Campylobacter*. Wild birds are known to harbor foodborne pathogens, including *Campylobacter* and *Salmonella (32, 33, 46)*, and fecal material from these animals can potentially contaminate vegetable crops *(29)*. Farmers from farm IV were using their own processed manure from livestock to make fertilizer. Untreated wastewater and animal and human feces were frequently considered sources of contamination *(10, 26)*. The availability and accessibility of wash and comfort stations in the farms also affected the farm workers' hygiene, which eventually affected the safety of the fresh produce *(59)*. The World Health Organization *(59)* stated that the presence of

TABLE 5. *Wet markets samples that were positive for* C. jejuni *and isolates that were positive for* cdt

			No. (%) of samples							
			PCR				mCCDA			
Location	Vegetable type[a]	No. of samples	*C. jejuni*	*cdtA*	*cdtB*	*cdtC*	*C. jejuni*	*cdtA*	*cdtB*	*cdtC*
Wet market I	A	24	8 (33.33)	ND[b]	1 (4.17)	6 (25.00)	3 (12.50)	ND	ND	ND
	B	20	12 (60.00)	1 (5.00)	1 (5.00)	10 (50.00)	7 (35.00)	ND	ND	4 (20.00)
	D	20	1 (5.00)	ND	ND	1 (5.00)	1 (5.00)	ND	ND	1 (5.00)
	F	20	11 (55.00)	ND	ND	ND	ND	ND	ND	ND
	H	20	17 (85.00)	ND	ND	17 (85.00)	ND	ND	ND	ND
	J	24	14 (58.33)	3 (12.5)	1 (4.17)	11 (55.00)	2 (8.33)	ND	ND	2 (8.33)
Total		128	63 (49.20)	4 (3.13)	3 (2.34)	45 (35.16)	13 (10.16)	ND	ND	7 (5.47)
Wet market II	A	2	ND	ND	ND	ND	ND	ND	ND	ND
	B	10	10 (100.00)	ND	ND	7 (70.00)	1 (10.00)	ND	ND	ND
	C	10	5 (50.00)	ND	ND	ND	ND	ND	ND	ND
	D	12	7 (58.33)	ND	ND	10 (83.30)	1 (8.33)	ND	ND	1 (8.33)
	E	3	ND	ND	ND	ND	ND	ND	ND	ND
	H	10	8 (80.00)	ND	ND	9 (90.00)	ND	ND	ND	ND
	J	13	6 (46.15)	ND	1 (7.69)	1 (7.69)	ND	ND	ND	ND
Total		60	36 (60.00)	ND	1 (1.67)	27 (45.00)	2 (3.33)	ND	ND	1 (1.67)

[a] A, winged bean; B, long yard bean; C, Indian pennywort; D, water spinach; E, cucumber; F, cabbage; H, wild cosmos; J, Japanese parsley.
[b] ND, not detected.

TABLE 6. *Farm samples that were positive for* C. jejuni *and isolates that were positive for* cdt

			No. (%) of samples							
			PCR				mCCDA			
Location	Sample type[a]	No. of samples	*C. jejuni*	*cdtA*	*cdtB*	*cdtC*	*C. jejuni*	*cdtA*	*cdtB*	*cdtC*
Farm I	A	15	1 (6.67)	ND[b]	ND	ND	ND	ND	ND	ND
	B	10	5 (50.00)	ND	ND	6 (60.00)	ND	ND	ND	ND
	K	20	ND	ND	ND	ND	ND	ND	ND	ND
	L	20	4 (20.00)	ND	ND	10 (50.00)	ND	ND	ND	ND
Total		65	10 (15.38)	ND	ND	16 (24.62)	ND	ND	ND	ND
Farm II	H	10	7 (70.00)	ND	ND	ND	ND	ND	ND	ND
	K	10	ND	ND	ND	ND	ND	ND	ND	ND
	L	20	3 (15.00)	ND	ND	ND	ND	ND	ND	ND
Total		40	10 (25.00)	ND	ND	ND	ND	ND	ND	ND
Farm III	H	10	3 (30.00)	ND	ND	ND	ND	ND	ND	ND
	J	10	ND	ND	ND	ND	ND	ND	ND	ND
	K	10	1 (10.00)	ND	ND	ND	ND	ND	ND	ND
	L	20	14 (70.00)	ND	ND	1 (5.00)	3 (15.00)	ND	ND	1 (5.00)
Total		50	18 (36.00)	ND	ND	1 (2.00)	3 (6.00)	ND	ND	1 (2.00)
Farm IV	E	10	5 (50.00)	ND	ND	1 (10.00)	ND	ND	ND	ND
	H	10	9 (90.00)	ND	ND	3 (30.00)	2 (20.00)	ND	ND	ND
	I	10	7 (70.00)	ND	ND	ND	2 (20.00)	ND	ND	ND
	K	10	4 (40.00)	ND	ND	ND	ND	ND	ND	ND
	L	10	5 (50.00)	ND	ND	ND	2 (20.00)	ND	ND	ND
Total		50	30 (60.00)	ND	ND	4 (8.00)	6 (12.00)	ND	ND	ND
Farm V	D	10	2 (20.00)	ND	ND	3 (30.00	ND	ND	ND	ND
	E	10	7 (70.00)	ND	ND	4 (40.00)	ND	ND	ND	ND
	H	10	6 (60.00)	ND	ND	ND	ND	ND	ND	ND
	K	10	4 (40.00)	ND	ND	ND	ND	ND	ND	ND
	L	10	3 (30.00)	ND	ND	ND	ND	ND	ND	ND
Total		50	22 (44.00)	ND	ND	7 (14.00)	ND	ND	ND	ND

[a] A, winged bean; B, long yard bean; D, water spinach; E, cucumber; H, wild cosmos; I, Vietnamese coriander; J, Japanese parsley; K, fertilizer; L, soil.
[b] ND, not detected.

sick workers or children in fields or packing facilities can affect the quality of fruits and vegetables.

Although the prevalence of *C. jejuni* in farms was lower than that in retail outlets, the health hazards associated with leafy green vegetables can be amplified as they move through the food chain. Among the food chain stages, primary production is probably the most important with regard to the introduction to health hazards, followed by postharvest contamination during transportation (e.g., open, unprotected transport), processing (mixing of different types of leafy greens), packing (contamination by handlers), distribution, and markets or retail (wet markets). These stages may also provide conditions for population increases in any contaminating pathogens *(59)*.

Antibiotic resistance among pathogens had become a worldwide problem that is both a threat to public health and an important food safety issue *(23)*. *C. jejuni* resistance to antibiotics had created concerns among researchers and consumers. Although numerous studies had been conducted to assess antimicrobial resistance in *Campylobacter* isolates of animal origin *(1–3, 25)*, isolates from vegetables have not been well studied *(15)*. Chai et al. *(15)* reported high resistance to quinolones in *Campylobacter* isolates from ulam samples. In contrast, the results of the present study indicate that *C. jejuni* isolates from vegetables were highly susceptible to enrofloxacin and ciprofloxacin (Table 7). The blame for antibiotic resistance in *C. jejuni* has been put on both clinical and agricultural use of antibiotics. Wilson *(58)* found that *Campylobacter* resistance to fluoroquinolones

TABLE 7. *Antimicrobial susceptibility test results for* Campylobacter jejuni *isolates obtained from various samples*

			No. (%) of samples	
Antimicrobial agent	Disk content	*n*	Resistant	Susceptible
Amikacin	30 μg	33	2 (6.06)	31 (93.94)
Ampicillin	10 μg	33	25 (75.76)	8 (24.24)
Ciprofloxacin	5 μg	33	0	33 (100.00)
Enrofloxacin	5 μg	33	0	33 (100.00)
Erythromycin	15 μg	33	20 (60.61)	13 (39.39)
Gentamicin	10 μg	33	0	33 (100.00)
Norfloxacin	10 μg	33	1 (3.03)	32 (96.97)
Penicillin G	10 IU	33	32 (96.97)	1 (3.03)
Tetracycline	30 μg	33	3 (9.09)	30 (90.91)
Vancomycin	5 μg	33	29 (87.88)	4 (12.12)

may be due to wide use of these antibiotics in poultry. In addition, fluoroquinolone-resistant *Campylobacter* strains in chickens outcompete the majority of fluoroquinolone-susceptible strains *(49)*. The use of fluoroquinolones as chemoprophylaxis in poultry has led to the high resistance *Campylobacter* isolates to fluoroquinolones *(19, 28, 31, 42, 47)*. Antimicrobial agents are not likely to be used in vegetable farming, which might explain the low resistance to quinolones found in the present study (Table 7).

Bang et al. *(5)* detected *cdt* genes (i.e., *cdtA, cdtB,* and *cdtC*) in *C. jejuni* isolates from various sources. The presence of *cdt* genes in *C. jejuni* isolates differs according to geographic origin *(35)*. Sahilah et al. *(44)* also detected low numbers of *Campylobacter* strains in vegetables that harbored *cdt* genes. However, the distribution of *cdtA, cdtB,* and *cdtC* genes in *C. jejuni* isolated from other sources, such as poultry, cattle, and pigs, were higher than 90.00% *(4, 5)*. Pickett et al. *(40)* suggested that some sort of mutation may have occurred within primer binding sites. In the present study, only nine isolates with *cdtC* were detected, which might be due to mutation of *cdtA* and *cdtB (35)*.

The detection of *C. jejuni* and *cdt* genes in this study indicates that salad vegetables can be contaminated and pose significant health risks to consumers. Good farming practices and proper management from farm to retail are important to control *Campylobacter* contamination.

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