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# Detection of virulence genes and enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) analysis among raw vegetables isolates of *Campylobacter jejuni*

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**Abstract**: A total of 20 (n=20) raw vegetable isolates of *Campylobacter jejuni* were examined for their virulence genes and genetic diversity by enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) analysis. All raw vegetable isolates of *C. jejuni* were encoded cadF and ceuE genes. None of the *C. jejuni* isolates was cdtA positive. Whereas, 12 (12/20) isolates were positive for cdtB and 6 (6/20) were positive for cdtC. All the 20 isolates of *C. jejuni* were subtyped and they produced 18 ERIC-PCR profiles namely E1 to E18. Dendrogram performed from cluster analysis showed that the 20 isolates were grouped into 2 major clusters, cluster I and II indicating the high level of local geographical genetic variation. The detection of virulence genes among *C. jejuni* strains isolated from raw vegetables raised concern on the threat of campylobacteriosis.

**Keywords: Virulence genes, ERIC-PCR, raw vegetables, *Campylobacter jejuni***

## Introduction

*Campylobacter jejuni* is a foodborne bacterium which can cause human gastroenteritis. The infection of these bacteria is mostly associated with poultry consumption particularly when handling raw poultry and eating undercooked poultry (Kapperud *et al*., 1992; Thomas *et al.,* 1997). The infection of these bacteria in sporadic case are also extended to drinking untreated water (Hopkins *et al.*, 1984), barbecued pork (Oosterom *et al*., 1984), raw milk (Schmid *et al.,* 1987), and contact with dog and cat (Kapperud *et al.,* 1992). The outbreaks of campylobacter illness associated with consumption of contaminated vegetables or fruits are uncommon, but possible (Centers for Disease Control, 2000).

There are several virulence genes that have been reported to describe the pathogenesis of *C. jejuni* infection in human. There are *fla*A gene encode for a flagella protein that is essential for motility, colonization of the gastrointestinal tract, and invasion of host cells *cad*F which is important in adhesion and colonization of the host's intestine (Nuijten *et al.*, 1992; Konkel *et al.,* 1999); *ceu*E gene encodes a binding-protein transport system for the siderophore enterochelin (Park and Richardson, 1995; Gonzales *et al.,* 1997), and cytolethal distending toxin (CDT) (*cdt*A, *cdt*B and *cdt*C) which are composed of *cdt*B as the enzymatically active subunit, and the two heterodimeric subunits *cdt*A and *cdt*C responsible for the holotoxin binding to cell membrane (Lara-Tejero and Galan, 2001). Specific PCR amplification of those genes in food (Fermer and Engvall, 1999) indicating a possible threat of campylobacteriosis when raw food being served to human.

The molecular typing based on PCR-based techniques have been developed to analyze the genetic diversity of *C. jejuni* and other bacteria, these includes enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) (Versalovic *et al.,* 1991; Milleman *et al.,* 1996), random amplified polymorphic DNA-PCR (RAPD-PCR) (Owen and Hernandez, 1993; Mehmet and Burhan, 2006), PCR ribotyping (Lagatolla *et al*., 1996) and PCR restriction fragment length polymorphism (PCR-RFLP) (Korolik *et al.,* 1995). Bacterial typing in *Campylobacter* using RAPD-PCR

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and ERIC-PCR have been shown ease to apply and high discriminatory power in strains differentiation (Mehmet and Burhan, 2006; Wilson *et al*., 2009). Bacterial typing is of great value in epidemiology of infectious disease because it helps to recognize the reservoir of infection, sporadic disease setting and recognized virulent clones are present in a set of strains (Eisenstein, 1990).

To date no data have been reported on the outbreak of *C. jejuni* neither poultry consumption nor raw vegetables in Malaysia. However, data has shown the prevalence of *C. jejuni* in raw vegetables and being eaten as *Ulam* as well as in poultry sources are rather alarming (Chai *et al.,* 2008; John *et al*., 2009; Usha *et al*., 2010). Consequently, the detection of virulence genes in raw vegetable isolates of *C. jejuni* indicating a potential threat of campylobacteriosis. Typing of *C. jejuni* is of considerable important in the surveillance of possible community foodborne outbreaks of campylobacteriosis. In this study we examined the presence of virulence genes and conducted the genetic diversity among raw vegetable isolates of *C. jejuni* by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) analysis.

## Materials and Methods

### Campylobacter jejuni *strains*

Twenty (n=20) raw vegetable isolates of Campylobacter jejuni (CJ1-CJ20) were obtained from the laboratory of Food Science and Biotechnology, University Putra Malaysia, Serdang, Selangor. All strains were isolated from 5 different raw vegetables including kangkung (Ipomea aquatica), pegaga (Cantella asiatica), kesum (Poligonum minus), kacang botol (Psophocarpus tetragonolobus), and tauge (Vigna radiata). The geographical location of all C. jejuni isolates were originated from Selangor area (Table 1).

### *Preparation of DNA for PCR and ERIC-PCR*

DNA extraction was done involving boiling, chilling and centrifugation (**Perera and Murray**, 2008). Prior the DNA preparation, the cells were grown onto CAMP agar medium at 42°C for 48 hours in an anaerobic jar. The cells were transferred into 1.5 ml of microfuge tube which contained 1.0 ml of sterile distilled water. The cells were vortexed and centrifuged at 12,000 rpm for 1 min. The supernatant was discarded. The pellet was then washed with 1.0 ml sterile distilled water and vortex. Then, it was boiled at 97°C for 10 min and immediately was frozen at -20°C for 10 min. The tube was centrifuged at 10,000 rpm for 3 min. The supernatant was used as a template for PCR amplification.

### *Detection of* cadF *and* ceuE *genes*

The multiplex detection of cad*F* and ceu*E* genes and PCR condition were conducted as method described by Konkel *et al.* (1999) and Gonzalez *et al*. (1997). Two pairs of primers were used which cadF-F2B (5'-TTGAAGGTAATTTAGATATG-3'), cadF-R1B (5'CTAATACCTAAAGTTGAAAC-3') , JEJ1 (5'-CCTGCTACGGTGAAAGTTTTGC-3'), and JEJ2 (5'-GATCTTTTTGTTTTGTGCTGC-3') designed by Konkel *et al.* (1999) and Gonzalez *et al.* (1997), respectively. The assay was performed in a 25 µl volume containing 5.0 µl of 5 × PCR buffer (100 mmol $l^{-1}$ Tris–HCl, 35 mmol $l^{-1}$ $MgCl_2$, 750 mmol $l^{-1}$ KCl, pH 8.8), 1.0 µl of 10 mmol $l^{-1}$ dNTPs (Promega, Madison, USA), 1.0 µl of 10 pmol $µl^{-1}$ of primer cadF-F2B, cadF-R1B, JEJ1 and JEJ2, 0.3 µl of 2.0 units of *Taq* DNA polymerase (Promega, Madison, USA), 12.20 µl of sterile ultrapure deionized water and 2.5 µl of 100 ng DNA template. A negative-DNA control was performed by adding 1 µl of sterile ultrapure deionized water, a positive control was performed by adding 1 µl of the DNA sample. Amplification was performed in personal Eppendorf thermal-cycler (Eppendorf, Germany) with a temperature program consisting of the initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 second, annealing for 1 min at 42 °C and polymerization at 72 °C for 40 second. Final elongation was at 72 °C for 3 min. The amplification products were analyzed by electrophoresis in a 1.0% agarose in 0.5X TBE (0.1 M Tris, 0.1 M Boric acid, 0.1 mM EDTA) at 90 V for 40 minutes. Gels were stained with ethidium bromide. The amplified fragments were visualized with UV transilluminator (Syngene, USA). The 100 bp DNA ladder (Vivantis) was used as a DNA size marker.

### *Detection of* cdtA, cdt B *and* cdtC *genes*

The detection of cytolethal distending toxin (CDT) of *cdt*A, *cdt*B and *cdt*C and PCR condition were used as described by Pickett *et al.* (1992). All raw vegetable isolates of *C. jejuni* were amplified for gene encoded of *cdt*A, *cdt*B and *cdt*C in three separated experiment (Table 2). The assay was performed in a 25 µl volume containing 5.0 µl of 5 × PCR buffer (100 mmol $l^{-1}$ Tris–HCl, 35 mmol $l^{-1}$ $MgCl_2$, 750 mmol $l^{-1}$ KCl, pH 8.8), 1.0 µl of

**Table 1.** Prevelence of *Campylobacter jejuni* and their location in Selangor

| Strain no. | Sample source | Location |
|---|---|---|
| CJ1 | Kangkung (*Ipomea aquatic*) | Supermarket A, Serdang |
| CJ2 | Kangkung (*Ipomea aquatic*) | Supermarket A, Serdang |
| CJ3 | Pegaga (*Cantella asiatica*) | Supermarket A, Serdang |
| CJ4 | Pegaga (*Cantella asiatica*) | Supermarket A, Serdang |
| CJ5 | Kangkung (*Ipomea aquatic*) | Supermarket A, Serdang |
| CJ6 | Kesum (*Poligonum minus*) | Supermarket A, Serdang |
| CJ7 | Kacang botol (*Psophocarpus tetragonolobus*) | Supermarket B, Putrajaya |
| CJ8 | Kesum (*Poligonum minus*) | Supermarket B, Putrajaya |
| CJ9 | Tauge (*Vigna radiata*) | Supermarket B, Putrajaya |
| CJ10 | Kesum (*Poligonum minus*) | Supermarket B, Putrajaya |
| CJ11 | Kacang botol (*Psophocarpus tetragonolobus*) | Supermarket B, Putrajaya |
| CJ12 | Tauge (*Vigna radiata*) | Supermarket B, Putrajaya |
| CJ13 | Tauge (*Vigna radiata*) | Supermarket B, Putrajaya |
| CJ14 | Kangkung (*Ipomea aquatic*) | Supermarket B, Putrajaya |
| CJ15 | Kacang botol (*Psophocarpus tetragonolobus*) | Wet Market, Serdang |
| CJ16 | Pegaga (*Cantella asiatica*) | Wet Market, Serdang |
| CJ17 | Pegaga (*Cantella asiatica*) | Wet Market, Serdang |
| CJ18 | Pegaga (*Cantella asiatica*) | Wet Market, Serdang |
| CJ19 | Pegaga (*Cantella asiatica*) | Wet Market, Serdang |
| CJ20 | Pegaga (*Cantella asiatica*) | Wet Market, Serdang |

**Table 2.** Primers of cytolethal distending toxin (CDT) (*cdt*A, *cdt*B and *cdt*C) with their sequences.

| Gene target | Primer | Sequence (5'-3') |
|---|---|---|
| *cdtA* | GNW | GGAAATTGGATTTGGGGCTATACT |
| | IVH | ATCACAAGGATAATGGACAAT |
| *cdtB* | VAT2 | GTTAAAATCCCCTGCTATCAACCA |
| | WMI-R | GTTGGCACTTGGAATTTGCAAGGC |
| *cdtC* | WMI-F | TGGATGATAGCAGGGGATTTTAAC |
| | LPF-X | TTGCACATAACCAAAAGGGAAG |

10 mmol $l^{-1}$ dNTPs (Promega, Madison, USA), 1.0 µl of 10 pmol $µl^{-1}$ primer (either *cdt*A, *cdt*B or *cdt*C), 0.3 µl of 2.0 units of *Taq* DNA polymerase (Promega, Madison, USA), 12.20 µl of sterile ultrapure deionized water and 2.5 µl of 100 ng DNA template. A negative-DNA control was performed by adding 1 µl of sterile ultrapure deionized water, a positive control was performed by adding 1 µl of the DNA sample. Amplification was performed in personal Eppendorf thermal-cycler (Eppendorf, Germany) with a temperature program consisting of the initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 second, annealing for 1 min at 42 °C and polymerization at 72 °C for 40 second. Final elongation was at 72 °C for 3 min. The amplification products were analyzed by electrophoresis in a 1.0% agarose in 0.5X TBE (0.1 M Tris, 0.1 M Boric acid, 0.1 mM EDTA) at 90 V for 40 minutes. Gels were stained with ethidium bromide. The amplified fragments were visualized with UV transilluminator (Syngene, USA). The 100 bp DNA ladder (Vivantis) was used as a DNA size marker.

*Enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR)*

The primer used were ERIC1R (5'-CACTTAGGGGTCCTCGAATGTA-3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') as described by Versalovic *et al*. (1991). PCR amplification reactions consisted of 25 µl volume containing 10 ng of genomic DNA, 2.5 µl 10x reaction buffer, 0.5 µl of 2 unit *Taq* DNA polymerase, 1.0 µl of 5 µM each of the forward and reverse primers, 2 µl of 1.5 mM $MgCl_2$, and 1 mM each of dNTP. Amplification was done using a Perkin Almer 2400 thermocycler (Perkin-Elmer, Norwalk, USA) as follows: 95°C for 7 min and 35 cycles of denaturation at 95°C for 30 seconds, annealing at 52 °C for 1 min, and extension at 72 °C for 5 min. Final elongation step was at 72 °C for 10 min at the end of 35 cycles. The amplification products were fractionated by electrophoresis through 1.0 % agarose gel in 1X TBE buffer (0.1 M Tris, 0.1 M boric acid, 0.2 mM EDTA), detected by staining with ethidium bromide and were visualized with UV transilluminator (Syngene, USA). The 100 bp DNA ladder (Vivantis) was used as a DNA size marker.

*Data analysis*

The banding patterns of individuals' strains were scored based on the presence or absence of the bands. The banding patterns scored were analyzed using the RAPDistance Package Software (Version 1.04) program and this was used to analyze the ERIC-PCR profiles. The scoring was made in the form of binary code with the score '1' indicating presence of band and '0' the absence of band. The data obtained were recorded and entered in the software CorelDRAW Graphic Suite X3 where a dendogram was produced for further analysis. Clustering was based on the unweighted pair of group average method (UPGMA) and was performed with the RAPDistance software.

## Results

*Detection of* cadF *and* ceuE *virulence gene by multiplex PCR*

The primers used in this present work as described by Konkel *et al.* (1999) and Gonzalez *et al.* (1997). Two pair of primers was used for multiplex PCR analysis against 20 *Campylobacter jejuni* isolates to detect *cad*F and *ceu*E virulence gene. Figure 1 and Table 3 showed the multiplex PCR profiles of the target gene in *C. jejuni* isolates. All *C. jejuni* isolates were *cad*F and *ceu*E positive, indicated by formation of 2 bands in the range of molecular weight of 400-1000 bp. The presence of 400 bp band on the agarose gel indicated the *cad*F virulence gene (Konke1 *et al.,* 1999). While, a fragment of 984 bp indicated the presence of *ceu*E virulence gene (Gonzalez *et al*., 1997).

*Detection of* cdtA, cdtB *and* cdtC *genes*

None *cdt*A gene (422 bp) (Martinez *et al.,* 2006) was detected when 20 raw vegetable isolates of *C. jejuni* were examined by PCR amplification using primers described by Pickett *et al*. (1996) (Table 3). As shown in Table 3, specific PCR amplification for *cdt*B gene indicated 12 (12/20(CJ1, CJ2, CJ5, CJ7, CJ10-CJ15, CJ17 and CJ 18) raw vegetable isolates of *C. jejuni* were *cdt*B positive with the bands formation of 531 bp in molecular weight (Martinez *et al*., 2006). Whereas, 8 isolates (8/20) (CJ3, CJ4, CJ6, CJ8, CJ9, CJ16, CJ19 and CJ20) were lacked of *cdt*B, indicated by no band formation. As shown in Table 3, 6 (6/20) raw vegetable isolates of *C. jejuni* were *cdt*C positive (CJ4, CJ6, CJ9, CJ10, CJ16 and CJ17) while the remainder were lacked of *cdt*C gene. The band formation of *cdt*C was 339 bp in molecular weight (Martinez *et al*., 2006). Table 3 summarized the detection of virulence genes in raw vegetables isolates of *C. jejuni*.

*ERIC-PCR analysis*

Twenty isolates of *Campylobacter jejuni* were used for ERIC-PCR analysis with a pair of consensus oligonucleotides as described

**Table 3.** Virulence genes detected in raw vegetables isolates of *Campylobacter jejuni*

| Strain no. | *cad*F | *ceu*E | Cytolethal distending toxin (CDT) | | |
|---|---|---|---|---|---|
| | | | *cdt*A | *cdt*B | *cdt*C |
| CJ1 | + | + | - | + | - |
| CJ2 | + | + | - | + | - |
| CJ3 | + | + | - | - | - |
| CJ4 | + | + | - | - | + |
| CJ5 | + | + | - | + | |
| CJ6 | + | + | - | - | + |
| CJ7 | + | + | - | + | |
| CJ8 | + | + | - | - | - |
| CJ9 | + | + | - | - | + |
| CJ10 | + | + | - | + | + |
| CJ11 | + | + | - | + | - |
| CJ12 | + | + | - | + | - |
| CJ13 | + | + | - | + | - |
| CJ14 | + | + | - | + | - |
| CJ15 | + | + | - | + | - |
| CJ16 | + | + | - | - | + |
| CJ17 | + | + | - | + | + |
| CJ18 | + | + | - | + | |
| CJ19 | + | + | - | - | - |
| CJ20 | + | + | - | - | - |

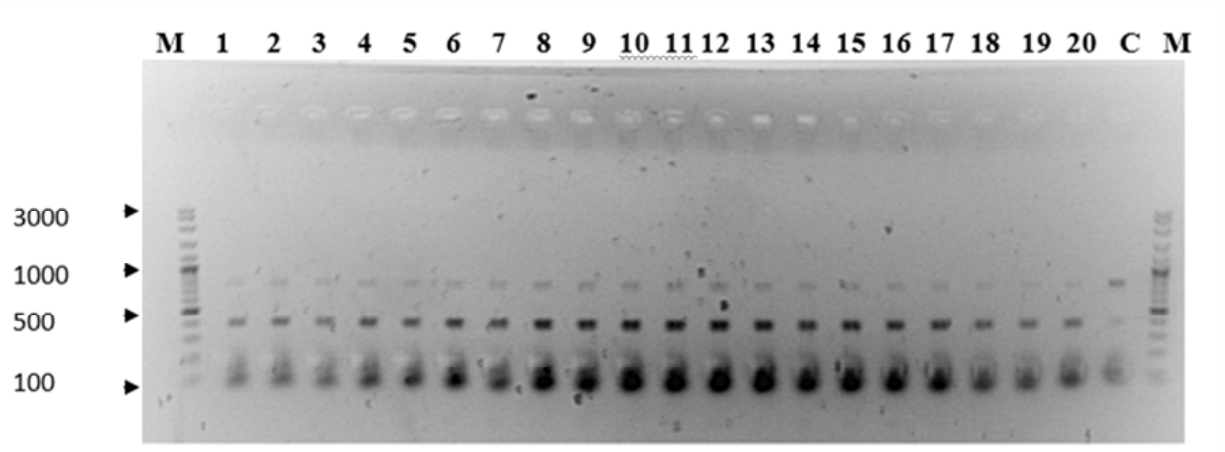


**Figure 1.** Multiplex detection of *cadF* and *ceuE* virulence gene among vegetable isolates of *Campylobacter jejuni* electrophoresed on 1.0 % agarose gel. Lane M, molecular weight sizes in base pairs (bp) are indicated by numbers on the left; lane 1-20: *C. jejuni* isolates CJ1, CJ2, CJ3, CJ4, CJ5, CJ6, CJ7, CJ8, CJ9, CJ10, CJ11, CJ12, CJ13, CJ14, CJ15, CJ16, CJ17, CJ18, CJ19 and CJ20; lane C was a positive control

by Versalovic *et al*. (1991). The ERIC-PCR genomic fingerprinting of *C. jejuni* revealed 18 distinct patterns (ERIC-PCR patterns from E1 to E18) (Figure 2, 3 and 4). The possible number of ERIC-PCR pattern was estimated on the basis of changes in one or more clear bands or band sizes. The number of ERIC-PCR bands produced for a given primer ranged from 1 to 12, with molecular sizes ranging from 0.1 to 3.0 kb.

*Unweighted pair group method with arithmetic mean (UPGMA)*

ERIC-PCR profiles were analysed using UPGMA. Figure 5 shows the dendrogram of ERIC-PCR of *C. jejuni* isolates. As shown in Figure 5, ERIC-PCR dendrogram devided into 2 major cluster, Cluster I and II. Cluster I, divided into 2 subclusters, subcluster Ia and Ib in which subcluster Ia contained the other 2 subclusters, IaA (CJ2, CJ6 and CJ16) and IaB (CJ9, CJ10, CJ13). Subcluster Ib contained *C. jejuni* strain CJ7, CJ17 and CJ 20. It was noted that the subcluster IaA was *C. jejuni* strains isolated from Supermarket A, IaB was *C. jejuni* strains isolated from wet market. Whereas, subcluster Ib was *C. jejuni* strains isolated from Supermarket B. Cluster II, divided into 2 subclusters, subcluster IIa and IIb. Subcluster IIa contained all *C. jejuni* strains isolated from supermarket A (CJ1, CJ3, CJ4 and CJ5). Whereas, subcluster IIb contained a mixture of *C. jejuni* strains isolated from supermarket B (CJ12, CJ8 and CJ11) and wet market (CJ15 and CJ19).

## Discussion and Conclusion

In this work, virulence genes and genetic diversity of 20 raw vegetable isolates of *Campylobacter jejuni* were examined by specific PCR amplification. All raw vegetable isolates of *C. jejuni* were encoded both *cad*F and *ceu*E genes. The *cad*F and *ceu*E genes are important virulence factors in pathogenesis of *C. jejuni* infection in human. The *cad*F gene was reported to be common in *C. jejuni* due to its important in adhesion and colonization (Nuijten *et al*., 1992; Konkel *et al*., 1999). Whereas, the *ceu*E is important because this gene encodes a binding-protein transport system for the siderophore enterochelin and its involvement in iron acquisition and bacterial infectivity (Park and Richardson, 1995; Gonzales *et al*., 1997). The *ceu*E gene is also highly conserved in *C. jejuni* neither in pathogenic or non-pathogenic within their respective species in human isolates (Gonzales *et al*., 1997). This may explain why all raw vegetable isolates of *C. jejuni* were *cad*F and *ceu*E genes positive. Both *cad*F and *ceu*E genes were also observed in Danish pigs and cattles (Bang *et al*., 2003).

In cytolethal distending toxin (CDT) of *cdt*A, *cdt*B and *cdt*C genes analysis, none of *cdt*A gene was detected in all raw vegetable isolates of *C. jejuni*. In *cdt*B and *cdt*C genes analysis, 12 *C. jejuni* isolates were encoded *cdt*B and 6 *C. jejuni* isolates were encoded *cdt*C gene. The CDT is a toxin produce by *C. jejuni* which can cause progressive cellular distention and ultimately death in Chinese hamster ovary (CHO) (Pickett *et al.,* 1999). In this study, the lacked of *cdt*A gene in all raw vegetables isolates of *C. jejuni* was inconclusive. In contrast, all CDT genes of *cdt*A, cdt*B* and *cdtC* genes were highly detected in *C. jejuni* isolated from Danish pigs and cattles (Bang *et al*., 2003).

The potential of ERIC-PCR analysis on genetic diversity of 20 *C. jejuni* isolated from raw vegetables has been demonstrated. The ERIC-PCR analysis was highly discriminated in distinguishing of 20 *C. jejuni* isolates into 18 distinct ERIC-PCR profiles. Thus, the raw vegetable isolates of *C. jejuni* examined in ERIC-PCR analysis exhibited high level of local geographical genetic variation. This result is in agreement with the wide heterogeneity of *C. jejuni* isolated from feces and carcasses (Wieczorek, 2009).

ERIC-PCR dendrogram was constructed with Unweighted Pair Group Method with Arithmetic mean (UPGMA). Dendogram performed from cluster analysis showed that all the 20 isolates of *C. jejuni* were clustered into two major cluster (I and II) in which each cluster were formed the other subcluster. Most of the cluster trends were formed according from where the *C. jejuni* were isolated. For example cluster Ia, though *C. jejuni* strains were isolated from supermarket A and wet market assemble in one cluster they were divided into two subcluster (IaA and IaB). While, cluster Ib was a group raw vegetable strains of *C. jejuni* isolated from supermarket B and cluster IIa was a group of raw vegetable strains of *C. jejuni* isolated from supermarket A. However, only cluster IIb was a mixture of *C. jejuni* strains isolated from raw vegetable obtained from supermarket B and wet market which may suggest *C. jejuni* clonal circulate within locations as indicated by 3 strains of *C. jejuni* (CJ8, CJ11 and CJ19).

No specific patterns have been shown in relation between virulence genes and its ERIC-PCR analysis among raw vegetable isolates of *C. jejuni*. The results of this study showed high level of local geographical genetic variation among the raw vegetable isolates of *C. jejuni* by ERIC-PCR analysis which may be useful in studying the epidemiology of *C. jejuni* isolates

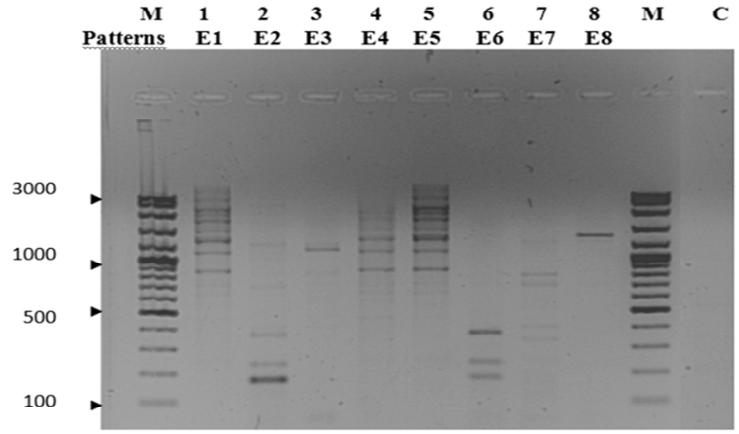


**Figure 2.** ERIC-PCR fingerprinting (E1 to E8) of *Campylobacter jejuni* isolates electrophoresed on 1.0% agarose gel. Lane M: 100 bp DNA ladder (molecular weight in bp); lane 1-8: samples number CJ1, CJ2, CJ3, CJ4, CJ5, CJ6, CJ7 and CJ8. Lane C: negative control

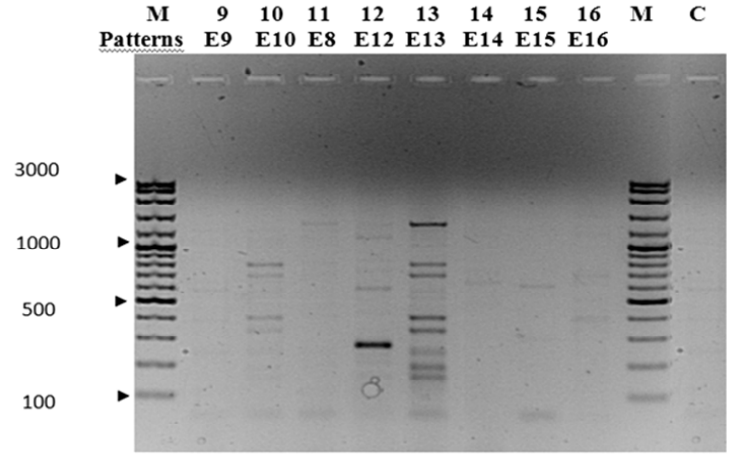


**Figure 3.** ERIC-PCR fingerprinting (E9 to E16) of *Campylobacter jejuni* isolates electrophoresed on 1.0% agarose gel. Lane M: 100 bp DNA ladder (molecular weight in bp); lane 9-16: samples number CJ9, CJ10, CJ11, CJ12, CJ13, CJ14, CJ15, CJ16. Lane C: negative control

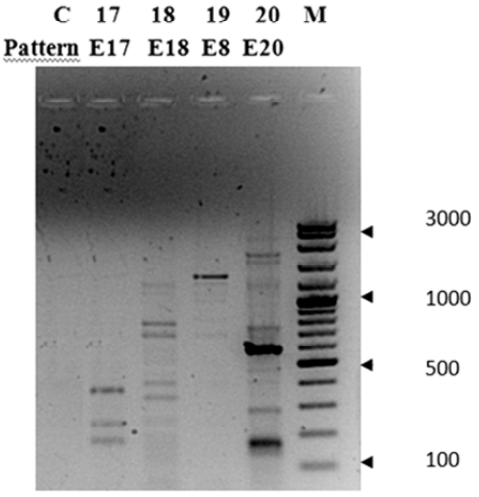


**Figure 4.** ERIC-PCR fingerprinting (E17 to E20) of *Campylobacter jejuni* isolates electrophoresed on 1.0% agarose gel. Lane M: 100 bp DNA ladder (molecular weight in bp); lane 17-20: samples number CJ17, CJ18, CJ19, CJ20. Lane C: negative Control

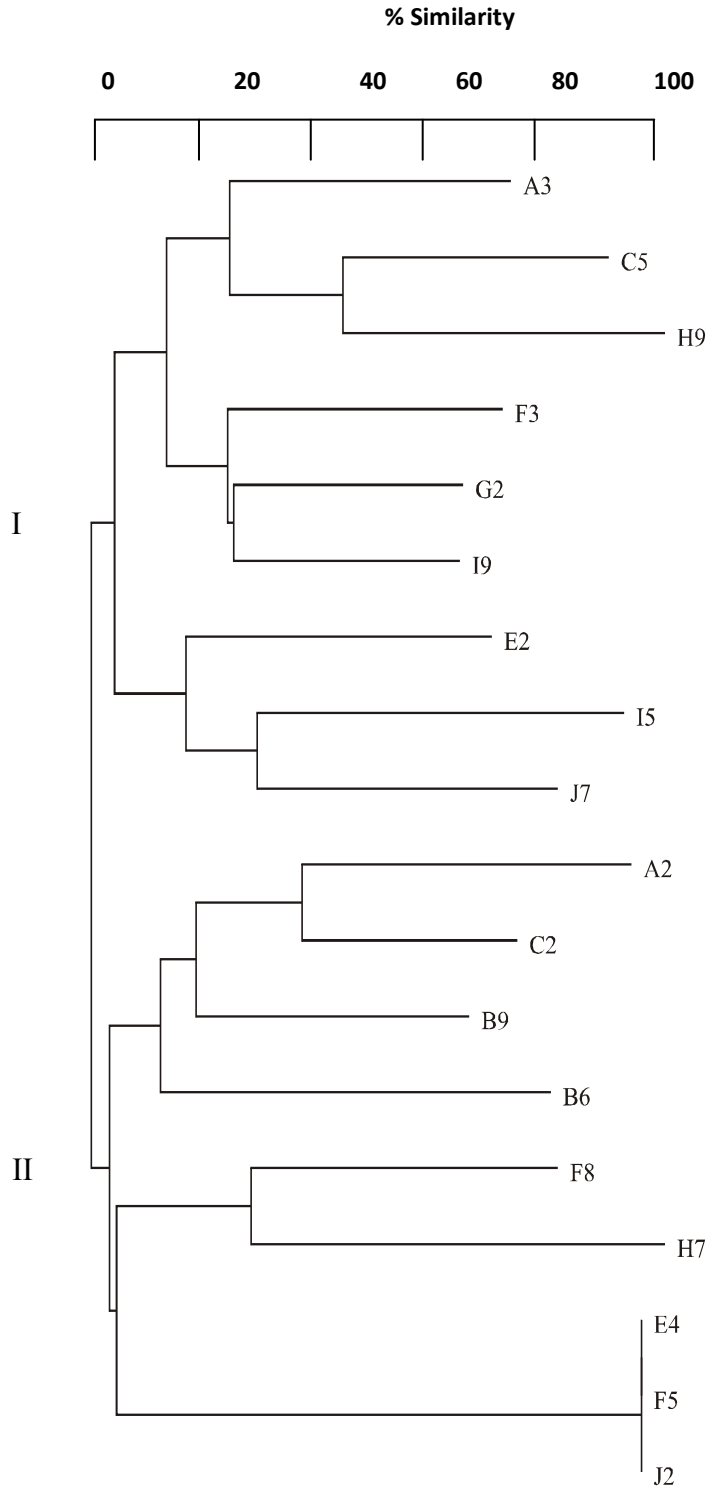


**Figure 5.** Dendogram generated from the ERIC-PCR profiles of *Campylobacter jejuni* isolates from raw vegetables

within local geographical locations. The detection of virulence genes in raw vegetable isolates of *C. jejuni* is rather concern due to some of the vegetables were eaten by Malaysian as *ulam* (salad) or raw vegetable which may potentially a threat of campylobacteriosis to the consumers.

## References


Bang, D.D., Moller Nielsen, E., Scheutz, F., Pedersen, K., Handberg, K. and Madsen, M. 2003. PCR detection of seven virulence and toxin genes of *Campylobacter jejuni* and *Campylobacter coli* isolates from Danish pigs and cattle and cytolethal distending toxin production of the isolates. Journal of Applied Microbiology 94 (6): 1003 – 1014.

Centers for Disease Control. 2000. Centers for Disease Control and Prevention. U. S. Foodborne Disease Outbreak Line Listing, 1990-1999. http://www.cdc.gov/ncidod/dbmd/outbreak/us_outb.htm. on 18/12/2006.

Chai, L. C., Fatimah, A. B., Ghazali, F. M., Lee, H. Y., Tunung, R., Shamsinar, A. T., Laila, R. A. S., Thahirahtul, A. Z., Malakar, P. M., Nakaguchi, Y., Nishibuchi, M. and Son, R. 2008. Biosafety of *Campylobacter jejuni* from raw vegetables consumed as Ulam with reference to their resistance to antibiotics. International Food Research Journal 15: 125-134.

Eisenstein, B. I 1990. New molecular techniques for microbial epidemiology and the diagnosis of infectious disease. Journal of Infectious Disease 160: 595-602.

Fermer, C. and Engvall, E. O. 1999. Specific PCR identification and differentiation of thermotolerant campylobacters, *Campylobacter jejuni, C. coli, C. lari and C. upsaliensis.* Journal of Clinical Microbiology 337: 3370-3376.

Gonzales, I. Grant, K.A. Richardson P.T.,. Park S.F and. Collins M.D, 1997. Specific identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* by using a PCR test based on the ceuE gene encoding a putative virulence determinant. Journal of Clinical Microbiology 35: 759–763.

Hopkins, R. S., Olmsted, R. and Istre, G. R. 1984. Endemic *Campylobacter jejuni* infection in Coloradi: identified risk factors. American Journal of Public Health 74: 249-250.

Kapperud, G., Skjerve, E., Bean N. H., Ostroff, S. M. and lassen, J. 1992. Risk factors for sporadic Campylobacter infections: results of a case-control study in southeastern Norway. Journal of Clinical Microbiology 30: 3117-3121.

Konkel, M.E., Gray, S.A., Kim, B.J., Garvis S.G. and Yoon, J. 1999. Identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* based on the cadF virulence gene and its products. Journal of Clinical Microbiology. 37, 510–517.

Korolik, V., Moorthy, L. and Coloe, P. J. 1995. Differentiation of *Campylobacter jejuni* and *C. coli* strains by using restriction endonuclease DNA profiles and DNA fragment polymorphisms. Journal of Clinical Microbiology 33: 1136-1140.

Lagatolla, C., Dolzani, L., Tonin, E., Lavenia, A., Michele, M. D., Tommasini, T. and Monti-Bragadin, C. 1996. PCR ribotyping for characterising Salmonella isolates of different serotypes. Journal of Clinical Microbiology 34: 2440-2443.

Lara-Tejero, M. and Galàn, J.E. 2001. *CdtA, CdtB*, and *CdtC* form a tripartite complex that is required for cytolethal distending toxin activity. Infection and Immunity 6: 4358–4365.

Lara-Tejero, M. and Galàn, J.E. 2002. Cytolethal distending toxin: limited damage as a strategy to modulate cellular functions. Trends in Microbiology 10: 147–152.

Martínez, I., Mateo, E., Churruca, E., Girbau, C., Alonso, R.and Fernández-Astorga, A. 2006. Detection of *cdtA, cdtB*, and *cdtC* genes in *Campylobacter jejuni* by multiplex PCR . International Journal of Medical Microbiology 296: 45-48.

Mehmet, N. A. and Burhan Ç. 2006. Random amplified polymorphic DNA analysis of *Campylobacter jejuni* and *Campylobacter coli* isolated from healthy cattle and sheep. Journal of Medical Microbiology 55: 331-334.

Milleman, Y., Lesage-Descauses, M. C, Lafint, J. P. and Chaslus-Dancle, E. 1996. Comparison of random amplified polymorphic-PCR DNA analysis and enterobacterial repitive intergenic consensus-PCR for epidemiological studies of *Salmonella*. FEMS Immunology and Medical Microbiology 33: 183-194.

Nuijten, P.J.M., Wassenaar, T.M., Newell D.G. and Van der Zeijst B.A. 1992. Molecular characterization and analysis of *Campylobacter jejuni* flagellin genes and proteins. In: I. Nachamkin, M.J. Blaser and L.S. Tompkins, Editors, *Campylobacter jejuni:* current status and future trends, pp. 282–296. American Society for Microbiology.

Oosterom, J., Den Uly., C. H., Banffer, J. R. J. and Huisman, J. 1984. Epedimiologic investigations on *Campylobacter jejuni* in householda with primary infection. Journal of Hygiene 92: 325-332.

Owen, R. J. and Hernandez, T. H. 1993. Chromosomal DNA fingerfrinting- a new method species and identification applicable to microbial pathogens. Journal of Medical Microbiology 30: 89-90.

Perera, K. and Murray, A. 2008. Development of a PCR assay for the identification of *Salmonella enterica* serovar Brandenburg. Journal of Medical Microbiology 57: 1223-1227.

Park, S.F. and Richardson, P.T. 1995. Molecular characterization of a *Campylobacter jejuni* lipoprotein with homology to periplasmic siderophore binding proteins. Journal of Bacteriology 177: 2259–2264.

Schmid, G. P., Schaefer, R. E., Plikaytis, B. D., Schaefer, J. R., Bryner, J. H. and Wintermeyer, L. A. 1997. A one-year study of endemic campylobacteriosis in a Midwestern city: association with consumption of raw milk. Journal of Infectious Disease 156: 218-222.

Tang, J.Y.H., Mohamad Ghazali, F., Saleha, A.A., Nishibuchi, M. and Son, R. 2009. Comparison of thermophilic *Campylobacter* spp. occurrence in two types of retail chicken samples. International Food Research Journal 16: 277-288.

Thomas, L. M., Long, K. A., Good, R. T., Panaccio, M. and Widders. 1997. Genotypic diversity among *Campylobacter jejuni* isolates in a commercial broiler flock. Applied and Environmental Microbiology 63: 1874-1877.

Usha, M. R., Tunung, R., Chai, L. C., Ghazali, F. M., Cheah, Y. K., Nishibuchi, M. and Son, R. 2010. A study on Campylobacter jejuni cross-contamination during chilled broiler preparation. International Food Research Journal 17: 107-115.

Versalovic, J., Koeuth, T. and Lupsi, J. R. 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic acids Research 19: 6823-6831.

Wieczorek, K. 2009. Relationship between the molecular typing of *Campylobacter* strains and the prevalence of their virulence genes. Bulletin of the Veterinary Institute in Pulawy 53: 193-198.

Wilson, M. K., Lane, A. B., Law, B. F., Miller, W. G., Joens, L. A., Konkel, M. E. and White, B. A. 2009. Analysis of the pan genome of *Campylobacter jejuni* isolates recovered from poultry by pulsed-field gel electrophoresis, multilocus sequence typing (MLST), and repetitive sequence polymerase chain reaction (rep-PCR) reveals different discriminatory capabilities. Microbial Ecology 58 (4): 843-55.