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MOLECULAR IDENTIFICATION OF *CAMPYLOBACTER JEJUNI* AND VIRULENCE GENES ISOLATED FROM DIARRHEIC COWS IN BAGHDAD, IRAQ

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ABSTRACT
The objective of this study was to investigate the 4 virulance genes from *Campylobacter jejuni* isolated from 1000 diarrheic cows in Baghdad governorate. A total of 30 *Campylobacter* isolates diagnosed as 30 *C. jejuni* were recovered from diarrheic cows by cultivation methods. The isolates were identified on the basis of polymerase chain reaction (PCR) detection of *16SrRNA* to determine three species from confirmed isolates, PCR was carried out for the presence of 4 virulence genes (*cadF*, *cdtA*, *cdtB*, and *Cdtc*) using specific primers. Results showed that: 30 suspected isolates of *Campylobacter spp* that were positive for o.b.i.s test were confirmed by PCR technique. The expected fragment approximately 1500bp of 16Sr na gene were successfully amplified as *Campylobacter jejuni* and 4 virulence gene (Cdta, Cdtb, and Cdtc and Cdaf) were present in equal distribution in all *Campylobacter jejuni* in ratio 10 (12.5%). Analyses Sequence of *C. jejuni* of this study showed all isolates successfully positive to sequences, all *Campylobacter* isolated strains showed 98- 99% similarity with reference strains in GenBank of *Campylobacter*.

KEYWORDS: *Campylobacter jejuni*, virulence genes, cows, Iraq.

INTRODUCTION

Campylobacter has become one of the most widely recognized causative agent of bacterial food borne gastroenteritis in human and healthy domestic and wild animals because the intestinal tracts of warm-blooded animals are a natural reservoir for *Campylobacter* spp. (Lindmark *et al*., 2004). It causes inflammation of the intestines which result in enteritis. The disease occoure sporadically in developed countries and more generally in undeveloped countries (Garenaux *et al*., 2008). The family Camplyobacteraceace includes more than 19 species and 9 subspecies (Kaakoush *et al*., 2015) The most species is a thermophlic, are *C. lari, C. coil and C. jejuni* (sallam, 2007). *Campylobacter* organisms produce two types of toxins: enterotoxin and cytotoxins. The enterotoxin of *C. jejuni* is similar to the *Vibrio cholerae* toxin and the *Escherichia coli* heat liable toxin, enterotoxin bind to cellular receptor, enter the cell and elevate intracellular cyclic adenosine mono-phosphate (cAMP) level. This enterotoxin is produced to a lesser degree by *C. coli.* It has been suggested that enterotoxin produced by *Campylobacter* spp results in watery diarrhea, as opposed to bloody diarrhea due to cytotoxin production. However, in some studies enter toxigenic strains have been isolated from asymptomatic carriers (Wassenaar, 1997). *Campylobacter* has several virulence-associated genes most of which associated with pathogenicity (Zilbauer *et al*., 2008). CadF is the expression of adherence and colonization (Ziprin *et al*., 2001), virB11 (Bacon *et al*., 2000) and pldA (Ziprin *et al*., 2001) are considerd as pathogenic genes responsible for the expression of invasion. The pathogenic gene responsible for expression of toxin production, are cdtA, cdtB, and *cdtC* (Lara-Tejero and Galan, 2001) waaC, wlaN and cgtB are genes that are presumably involved in the expression of ganglioside mimics in Guillian-Barré syndrome (Linton *et al*., 2000) .Virulence gene linked with Campylobacter invasiveness is the invasion-associated marker (iam) gene (Carvalho *et al*., 2001) CDT composed of three subunits encoded by the *cdt*A, *cdt*B *and cdt*C genes, causes eukaryotic cells death preventing them from entering mitosis and consequently leading to cell death(Ge *et al*., 2008).

MATERIALS & METHODS

Isolation and Identification

A total of 30 *Campylobacter* isolates diagnosed as 30 *C. jejuni* recovered from 1000 diarrheic cows faececs during May 2016 to July 2017 ,Fecal Samples were collected within 24 h. 1 g of each sample was was enriched in ml of Preston broth (nutrient broth no. 2 CM0067B, *Campylobacter* selective supplement SR0117E and lyzed horse blood SR0048, Oxoid) Incubation of Preston broth for 24 to 48 h at 42°C took place under microaerophilic conditions by using (Campygen, CN0025 or CN0035, Oxoid) and then made serial dilution in Peptone Water (CM0009B, Oxoid) . Following this, 0.1 ml of each serial dilution was streaked onto an mCCDA plate (modified Charcoal Deoxycholate Agar, *Campylobacter* blood-free selective medium, CM0739 and CCDA selective supplement, SR0155, Oxoid) and was incubated for 24 to 72 h at 42 °C in a microaerophilic atmosphere conditions (Ghafir *et al*., 2007).

Identification method

One presumptive *Campylobacter* colony from each selective agar plate was sub cultured onto Columbia blood agar (Oxoid) and incubated for 24 h under the same

conditions for the selective agar and tested by standard microbiological and biochemical procedures**,** differentiated at species level by Gram stain, oxidase and catalase activities, hippurate hydrolysis, TSI, hydrogen sulfide production and susceptibility to nalidixic acid by using a commercially available species differentiation kit (vitek nh compact 2 bioMérieux, Marcy-l'Etoile, France and oxoid biochemical identification system campy (O.B.I.S oxoid England).

DNA preparation

Three to five colonies suspected as *Campylobacter* were selected from each plate and sub-cultured onto Muller-Hinton agar plates, after which DNA was extracted from each isolate using the Genomic DNA Purification Kit The DNA was then quantified using the NanoDrop 1000 Spectrophotometer (Fisher Scientific, Pittsburgh, PA) and subjected to species-specific PCR analysis to confirm the identity of the isolates as described elsewhere (Denis *et al*., 2008). DNA from bacterial isolates was extracted by standard molecular biological techniques using the kit: Genomics DNA Purification (G _spin dna extraction kit , intron biotechnology was done according to the manufactures procedure.

PCR of genes targeted a 16S rRNA and virulence genes

The DNA from all isolates was amplified by PCR as a control for DNA extraction and *C. jejuni* confirmation by analysis of the 16SrRNA and Cdta, Cdt, Cdtc. Cdaf genes, Amplification of these genes were carried out in a master mix volume of 25ul containing (5ul Taq PCR Premix G _SPIN kit) and 10 picomols/µ (1ul) Forward primer and10 picomols/µ (1ul) Reverse primer DNA (1.5µl), Distill water (16.5µl) under optimal condition on table (2) The PCR products were separated by 2.% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302nm) after red stain staining (Intron Korea). The product was electrophoresis on 2.5% agarose at 5volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

Sequences of PCR products

10 PCR products of *C. jejuni* of *16srna gene* were purified using the G _spin dna extraction kit, intron biotechnology. The PCR products were automatically sequenced in one direction. Sequence alignment using Blast and Bio edit was used to sequence and the results were compared and matched with the database obtained from gene bank which is available at NCBI online for bioinformatics analysis as in table (1).

TABLE 1: The specific primer of genes (16srna, cdta, cdtb, cdtc, cdaf)

gene	Primer	Sequence	Tm (°C)	GC (%)	Product size
16Srna	Forward	5'-TTG ATC CTG GCT CAG AGT-3	52.8	50.0 %	1500
	Reverse	5'-TTC ACC CCA GTC GCT GAT-3	54.6	43.2 %	base pair
Cdta	Forward	5'-CCTTGTGATGCAAGCAAT-'3	52.2	47.4%	370 bp
	Reverse	5'- ACACTCCATTTGCTTTCTG-'3	50.6	2.1%	
cdtB	Forward	5'-CAGAAAGCAAATGGAGTGTT-'3	51.1	40.0%	620bp
	Reverse	5'-AGCTAAAAGCGGTGGAGTAT -'3	53.7	45.0%	
Cdtc	Forward	5'-CGATGAGTTAAAACAAAAGATA-'3	46.6	27.3%	182 bp
	Reverse	5'- TTGGCATTATAGAAAATACAGTT-'3	48.2	26.1%	
Cdaf	Forward	5'-TTGAAGGTAATTTAGATATG-'3	42.0	25.0%	400 bp
	Reverse	5'CTAATACCTAAAGTTGAAAC-'3	43.3	30.0%	

TABLE 2: The optimum condition of detection (16srna, cdta, cdtb, cdtc, cdaf)

gene	Phase	Tm (°C)	Time	No. of cycle
16srna	Initial Denaturation	94°C	3 min.	35cycle
	Denaturation	94°C	1 min	
	Annealing	52;°C	1min	
	Extension-1	72°C	1:40	
	Extension -2	72°C	10 min.	
Cdta	Initial Denaturation	94°C	3 min.	35cycle
	Denaturation	94°C	30 sec	
	Annealing	49°C	30sec	
	Extension-1	72°C	30sec	
	Extension -2	72°C	7 min.	
Cdtb	Initial Denaturation	94°C	3 min.	35cycle
	Denaturation	94°C	35sec	
	Annealing	51°C	35sec	
	Extension-1	72°C	35 sec	
	Extension -2	72°C	7 min.	
Cdtc	Initial Denaturation	94°C	3 min.	35cycle
	Denaturation	94°C	30 sec	
	Annealing	48°C	30sec	
	Extension-1	72°C	30sec	
	Extension -2	72°C	7 min.	
Cdtc	Initial Denaturation	94°C	3 min.	35cycle
	Denaturation	94°C	30 sec	
	Annealing	48°C	30sec	

	Extension-1	72°C	30sec	
	Extension -2	72°C	7 min.	
Cdaf	Denaturation	94°C	3 min.	35cycle
	Annealing	94°C	35 sec	
	Extension-1	45°C	35sec	
	Extension -2	72°C	35sec	
	Denaturation	72°C	7 min.	

RESULTS

PCR

The 30 suspected isolates were positive for o.b.i.s test of campylobacter spp, figure (1) which was confirmed by PCR technique. Amplification of bacterial genomic DNA was conducted by using two primers as mentioned in materials and methods. The best amplification of 16srRNA genes were observed at 94°C Denaturation, 52°C annealing, Extension 72°C temperature respectively, while the best amplification of Cdta gene were observed at 94°C Denaturation 49°C annealing Extension 72°C. The best amplification (cdtB, Cdtc, Cdaf) annealing 51°C, 48°C and 48°C respectively while Denaturation and Extension was 94°C and 72°C in all 4 virulence genes these Under optimal conditions, the expected fragment approximately 1500bp of 16srRNA gene were successfully amplified as *Campylobacter jejuni* as shown in figure (2) in the other hand the expected fragment or product size of (Cdta, CdtB, Cdtc ,Cdaf) virulence genes approximately (37, 620, 182 and 40bp) respectively as shown in figure (3,4,5,6).

Detection of virulence genes by PCR

Cow's isolates show the presence all virulence genes in different ratio. *jejuni* recovered from cows fecal samples the prevalence of *cdtA cdtB*, *cdtC* and Cdaf virulence genes were 10 (33.3%), 10 in *C. jejuni* in product size (370, 620, 400 and 180 bp) respectively figure (3,4,5,6) Detailed results of the PCR detection of the 4 virulence genes and 16srna gene in the *Campylobacter jejuni* obtained from cows fecal samples shown in Tables (3).

TABLE 3: Occurrence of4virulence genes results of *Campylobacter jejuni* isolated from cow faecal samples

Species	No	No. of cows isolates positive for virulence genes (%)			
		cdtA	*cdtB*	*cdtC*	*cadF*
Campylobacter jejuni	30	10(33.3%)	10(33.3%)	(%.33%) 10	(33.3%) 10

No significant difference at (P=0.40) Chi Square 2.94

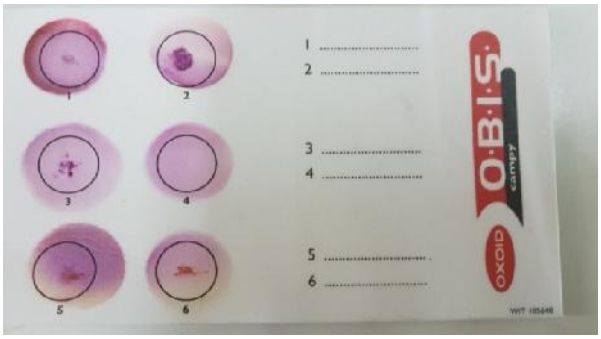

FIGURE 1: O.B.I.S(oxoid biochemical identification system campy 3,4.5 no colorless of colonies indicate the organism is campylobacter spp

.

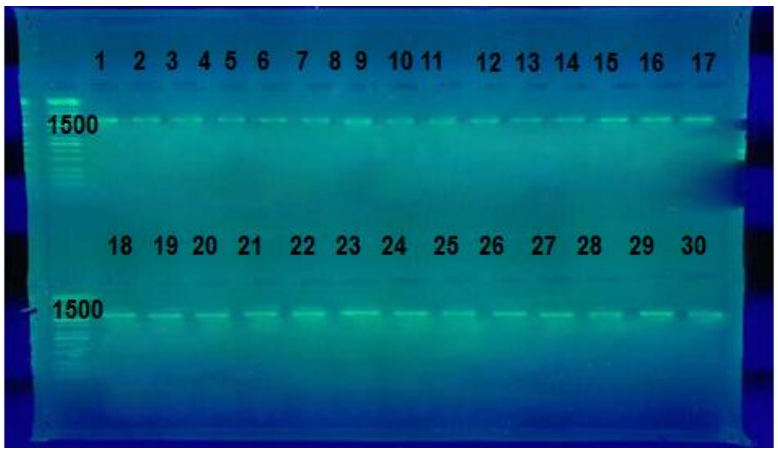

FIGURE 2: PCR product the band size 150016SrRNA. The product was electrophoresed on 1.5% agarose at 70 volt/cm^2. 1x TBE buffer for 1:30 hours N: DNA ladder (100), lane (1-30) represent (*Campylobacter jejuni)*.PCR product of band size 1500bp, visualized under U.V light

FIGURE 3: PCR product the band size 370 bp. The product was electrophoresed on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder (100), lane (1-10) represent gene Cdta

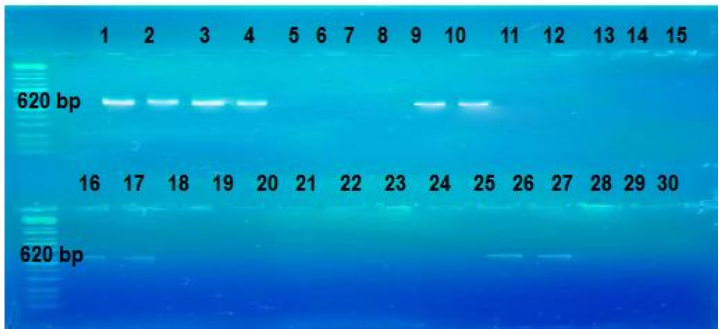

FIGURE 4: PCR product the band size 620 bp. The product was electrophoresed on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder (100), lane (1-10) represent gene cdtB

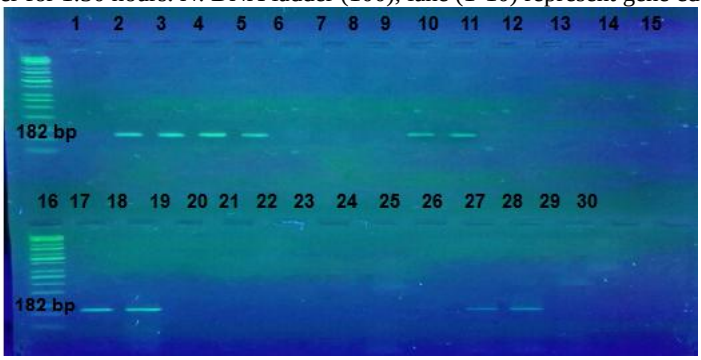

FIGURE 7: PCR product the band size 182bp. The product was electrophoresed on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours. N: DNA ladder (100), lane (1-10) represent gene Cdtc

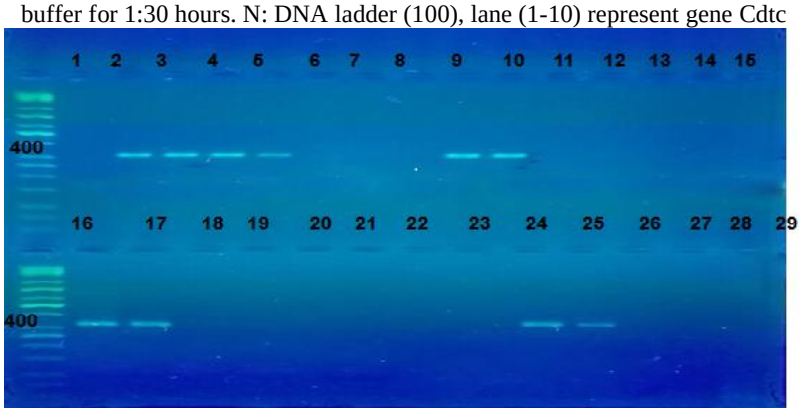

FIGURE 5: PCR product the band size 400bp. The product was electrophoresed on 2% agarose at 5 volt/cm^2 1x TBE buffer for 1:30 hours. N: DNA ladder (100), (1-10)represent gene, Cdaf

Analysis of the Nucleotide Sequence of Partial 16s rRNA Gene of campylobacter spp

Analyses Sequence of *C. jejuni* was done; the 1500bp fragment of 30 isolates of *C. jejuni* 16S rRNA gene was sequenced. The results showed 10 isolates of *C. jejuni* were successfully positive to sequences, Sequence alignment using Blast and Bio edit showed that all *Campylobacter* isolated strains showed 99% similarity, there is one insertion A(**▼A)** at position 602 score 1557, 1552 and there is one insertion A **(▼A**) at position 602 and other insertion G(**▼G)** at position 604 . Also there is 13 Transversion mutation 8 was (**C>A**), one was **(T>G)** and one was (**A>T)** and 4Transition (**G>A**) Our results were compared with data obtained from gene bank which is available at NCBI online Table (4).

TABLE 4: Result of sequence analysis of *Campylobacter jejuni* strains

No. of sample	Type of substitution	Location	Nucleotide	Range of nucleotide	Sequence ID	Score	Expect	Identities	Source
1	Transversion	281	C>A	26 to 924	ID: KT767834.1	1644	0.0	99%	*C. jejuni*
	insertion	602	▼A						
	Transversion	800	T>G						
2	Transversion	281	C>A	26 to 924	ID: KT767834.1	1644	0.0	99%	*C. jejuni*
	insertion	602	▼A						
3,4	Transversion	281	C>A	25 to 973	ID: KT767834.1	1731	0.0	99%	*C. jejuni*
	insertion	604	▼G						
	Transversion	799	T>G						
	Transition	960	G>A						
5	Transversion	281	C>A	19 to 917	ID: KT767834.1	1650	0.0	99%	*C. jejuni*
	Transversion	281	C>A	27 to 973	ID: KT767834.1	1722	0.0	99%	*C. jejuni*
	Transversion	608	A>T						
6	Transition	800	T>C						
	Transition	962	G>A						
	Transition	964	G>A						
7	Transversion	281	C>A	27 to 875	ID: KT767834.1	1557	0.0	99%	*C. jejuni*
	insertion	602	▼A						
8	Transversion	281	C>A	26 to 874	KT767834	1552	0.0	99%	*C. jejuni*
	insertion	602	▼A						
	Transversion	799	T>G						
	Transversion	225	T>A	26 to 873	KT767834	1550	0.0	99%	*C. jejuni*
9,10	Transversion	281	C>A						
	Transversion	799	T>G						

DISCUSION

In each of the 30 isolates, the *16Sr*RNA gene was detected by PCR. Thus, all isolates could be confirmed as *Campylobacter jejuni*, PCR product of the band size 1500bp. On 16SRNA figure (2) *16Sr*RNA gene was first genes tested in this study and this gene has been utilized extensively for rapid detection and identification of *Campylobacter* species (Kulkarni *et al*., 2002) This is can be attributed to the fact that the 16S rRNA gene is considerable length (1,500 bp), and it is ubiquitous in members of the *Campylobacter* genus and almost all bacteria (Clarridge, 2004). The outcome of PCR product 1500 bp of 16S rRNA gene in our study in all bacterial isolates figure (2), The second genes detected were cytolethal distending toxin (CDT) associated genes. Cytolethal distending toxin (CDT) is widely distributed among Gram-negative bacteria (Ceelen *et al*., 2006) and is best characterized of the toxins produced by *Campylobacter* spp. It has been described as an important virulence factor of this pathogen (Asakura *et al*., 2008) The CDT of *Campylobacter*, encoded by the *cdtA*, *cdtB*, and *cdtC* genes, damages host enterocytes and makes the penetration of the intestinal epithelium possible. Another gene which was tested in the present study was the *cadF* gene, an adhesion and fibronectin binding protein involved in the process of invasion, influencing microfilament organizations in host cells (Monteville *et al*., 2003). The CdtA, CdtB CdtC and gene Cdaf present in equal distribution in all *Campylobacter jejuni i*n ratio 10 (12.5%) in product size (370, 620, 180 and 400 bp) figure (3,4,5,6). Analysis of the prevalence of the *cadF*, *cdtA*, *cdtB*, and *cdtC* genes among *Campylobacter* isolates revealed that not all *Campylobacter* isolates carried these genes and indicate there is relationship between these virulence genes and clinical severity in bacterial isolates and play important role pathogenesis of campylobacteriosis. The genetic similarity between isolates may reflect infection come from same source similar results supporeted by) Samosornsuk *et al*., 2007). who noticed that The *cdt* gene clusters are ubiquitously distributed in *C. jejuni*, *C. coli* and *C. fetus* Similar observations have indicated the high prevalence of these genes in Similar study found in Chilelian study who observed *cdt*B gene was present in 100% of *C. jejuni* isolates (González-Hein *et al*., 2013). Our results also agreement with Khoshbakht *et al.* (2013) who found that genes *cadF* and *cdtA*, *cdtB*, and *cdtC* are present in thermophilic *Campylobacter* isolates but disagreement with him in isolation rate. In our study we have found that the type strains of *Campylobacter* jejuni exhibit 99% similarity with reference strains in GenBank, Table (4) and these species are clearly distinguishable biochemically and morophology and by o.b.i.s and this indicate that sequence similarity to a very high level Our results were compared with data obtained from gene bank which is aviliable at NCBI online. This high sequence similarity observed between members of the *Campylobacter* genus also makes it difficult to differentiate between

Campylobacter species on the basis of the 16S rRNA gene \ (On, 2001.) This is supported by Gorkiewicz *et al.* (2003) who noticed in the case of *C. jejuni*, *C. coli* and *C. lari* differentiation by the *16S rRNA* method is not sufficiently discriminating given the high degree of similarity found between the *16S rRNA* gene sequences for these three species. The fact that certain regions of the 16S rRNA gene are highly conserved, and that any changes in the sequence are therefore likely to be an accurate measure of time, makes it a useful molecular marker for the study of phylogenetic relationships (Janda and Abbott, 2007). Although 16S rRNA gene sequencing is highly useful in regards to bacterial classification, it has low phylogenetic power at the species level and poor discriminatory power for some gener (Mignard and Flandrois, 2006).

REFERENCES

Asakura, M., Samosornsuk, W., Hinenoya, A., Misawa, N., Nishimura, K., Matsuhisa, A. and Yamasaki, S. (2008) Development of acytolethal 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, 260–26.

Bacon, D.J., Alm, R.A., Burr, D.H., Hu, L., Kopecko, D.J., Ewing, C.P., Trust, T.J. and Guerry, P. (2000) Involvement of a plasmid in virulence of *Campylobacter jejuni* 81-176. Infect. Immun., 68: 4384-4390.

Carvalho, A.C.T., Ruiz-Palacios, G.M., Ramos-Cervantes, P., Cervantes, L.E., Jiang, X. and Pickering, L.K/ (2001) Molecular characterization of invasive and noninvasive *Campylobacter jejuni* and *Campylobacter coli* isolates. J. Clin. Microbiol., Vol. 39 :. 4 p. 1353–1359

Clarridge, J. E. (2004) Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clin. Microbiol. Rev. 17:840–862.

Ceelen, L., Decostere, A., Ducatelle, R. and Haesebrouck, F. (2006) Cyto-lethal distending toxin generates cell death by inducing a bottleneckin the cell cycle. *Microbiol. Res.* 161, 109–120.

Gorkiewicz, G., Feierl, G., Schober, C. (2003) Species-specific identification of campylobacters by partial 16S rRNA gene sequencing. J Clin Microbiol., 41: 2537-46.

Denis, M., Rose, V., Huneau-Salaün, A., Balaine, L. and Salvat, G. (2008) Diversity of pulsed-field gel electrophoresis profiles of *Campylobacter jejuni* and *Campylobacter coli* from broiler chickens in France. Poult. Sci. 87: 1662-1671.

Ge, Z., Schauer, D.B. & Foz, J.G. (2008) *In vivo* virulence properties of bacterial cytolethal-distending toxin. *Cell. Microbiol.* 10, 1599–1607..

Garenaux, A., Luchetti_Miganeh, C., Ermel, G., Barloy Hubler Frederique, D., Newell, D., Payot, S., Guillou, S. and Magaliritz (2008): Butter understanding of campylobacter conundrum Nova Science Publishers, Inc. Newyork.

Ghafir, Y., China, B., Dierick, K., De Zutter, L. and Daube, G. (2007) A seven-year survey of *Campylobacter* contamination in meat at different production stages in Belgium. International Journal of Food Microbiology, 116:111–120.

Gonzalez-Hein, G., García, P., Foerster, C., Figueroa, G. (2013) Prevalence of virulence genes in strains of *Campylobacter jejuni* isolated from human, bovine and broiler Brazilian Journal of Microbiology 44, 4, 1223-1229.

Janda, J.M. & Abbott, S.L. (2007) 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. J. Clin. Microbiol. 45:2761–2764.

Kaakoush, N.O., Natalia Castao-Rodrguez, Hazel, M., Mitchell, A. Ming (2015) Global Epidemiology of *Campylobacter* Infection Clinical Microbiology Reviews V: 28 .N: 3 .p :688

Kulkarni, S.P., Lever, S., Logan, J.M., Lawson, A.J., Stanley, J. and Shafi, M.S. (2002) Detection of *Campylobacter* species: a comparison of culture and polymerase chain reaction based methods. J. Clin. Pathol. 55:749–753.

Khoshbakht, R., Tabatabaei, M., Shirzad Aski, H. and Hosseinzadeh, S. (2013) Occurrence of virulence genes and strain diversity of thermophilic campylobacters isolated from cattle and sheep faecal samples Iranian Journal of Veterinary Research, Shiraz University IJVR, 2014, Vol. 15, No. 2, Ser. No. 47, p: 138-144.

Lara-Tejero, M. and Galan, J.E. (2001) *CdtA*, *CdtB*, and *CdtC* form .a tripartite complex that is required for cytolethal distending toxin activity. Infect. Immun., 69: 4358-4365.

Linton, D., Gilbert, M., Hitchen, P.G., Dell, A., Morris, H.R., Wakarchuk, W.W., Gregson, N.A. and Wren, B.W. (2000) Phase variation of a b-1,3 galactosyltransferase involved in generation of the ganglioside GM1-like lipooligosaccharide of *Campylobacter jejuni*. Mol. Microbiol., 37: 501-514.

Lindmark, H., Harbom, B., Thebo, L., Andersson, L., Hedin, G., Osterman, B., Lindberg, T., Andersson, Y., Westöö, A., Olsson Engvall, E. (2004) Genetic Characterization and Antibiotic Resistance of *Campylo-bacter jejuni* isolated from meats, water, humans in Sweden. J. Clin. Microbiol. 42: 700-706.

Monteville, M.R., Yoon, J.E., KonkelL, M.E. (2003) Maximall adherence and invasion of INT 407 cells by *Campylobacter jejuni* requires the *CadF* outer membrane protein and microfilament reorganization Microbiology, Reading, v. 149, n. 1, p. 153–165, 2003.

Mignard, S. and Flandrois, J.P. (2006) 16S rRNA sequencing in routine bacterial identification: a 30-month experiment. J. Microbiol. Methods 67:574–581.

Sallam, K. (2007) Prevalence of Campylobacter in chicken and chicken by-products retailed in Sapporo area, Hokkaido, Japan. Food Control, 18: 1113-1120.

Samosornsuk,W., Asakura, M., Yoshida, E., Taguchi, M., Kazuhiko, N., Boonchuay, E., Vongsavanh, P., Wan- pen, C. and Shinji, Y. (2007) Evaluation of a cytolethal distending toxin (*cdt*) gene-based species- specific multiplex PCR assay for the identification of *Campylobacter- ter* strains isolated from poultry in Thailand. *Microbiol. Immunol.* 51, 909–917.

Zilbauer, M., Dorrell, N., Wren, B.W. and Bajaj-Elliott, M. (2008) *Campylobacter jejuni*-mediated disease pathogenesis: an update. Trans. R. Soc. Trop. Med. Hyg., 102: 123-129

Ziprin, R.L., Young, C.R., Byrd, J.A., Stanker, L.H., Hume, M.E., Gray, S.A., Kim, B.J. and Konkel, M.E. (2001) Role of *Campylobacter jejuni* potential virulence genes in cecal colonization. Avi. Dis., 45: 549-557

On, S.L. (2001) Taxonomy of *Campylobacter*, *Arcobacter*, *Helicobacter* and related bacteria: current status, future prospects and immediate concerns. Symp. Ser. Soc. Appl. Microbiol. 30:1S–15S.