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Review article

Detection Important Virulence Factor (Cytolethal Distending Toxin gene) In *Campylobacter Jejuni* from Chicken By Real-Time PCR Technique

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ABSTRACT: Campylobacteriosis is a globally extended distributed zoonotic diseases , cytolethal distending toxin (CDT) is one of the main virulence elements related to *Campylobacter jejuni* pathogenesis in human and animal species . The contamination of poultry carcasses in slaughterhouses and consequent consumption or handling of raw or undercooked meat is most significant risk factors. In this study we used Real-Time PCR based SYBER Green dye amplification as advance molecular technique in direct detection cytolethal distending toxin gene in genomic DNA that extracted from broiler chicken stool samples, the specific primers was designed in this study by using NCBI-Gen Bank data base and primer 3 plus. Real-Time PCR results were show high occurrence of *Campylobacter jejuni* that carrying virulence factor (Cytolethal distending toxin) in stool samples of broiler chicken. Out of 50 chicken stool samples (41) positive samples at (82%). In conclusion the present study was concluded that shedding of *C. jejuni* that contains to cytolethal distending toxin (CDT) contributed very important risk factors to public health , use of Real-Time PCR technique is fast and very specific molecular technique.

KEYWORDS: Real-Time PCR technique, *Campylobacter jejuni*, chicken, cytolethal distending toxin, SYBER Green dye.

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INTRODUCTION

The food-borne bacterial pathogen *Campylobacter jejuni* has been reported in general within mainly developed countries[1] its microaerophilic, spiral or curved bacterium, gram negative, that cause a self-limiting watery with or without blood diarrhea in human[2]. The mucus of the ceca and the small intestine in chicken are colonized by *C jejuni*, and they can also be recovered from liver and spleen[3]. In the last 30 years the organism has gained more priority and it also has been known as a main cause of human diseases ranging from gastroenteritis to Guillain-Barre Syndrome[4,5]. The handling and consumption of infected meat especially chicken meat is supposed to be the major source of *C. jejuni* in humans[6] Bacterial toxins have been accounted important factors for the pathogenesis of Campylobacter disease, The cytolethal distending toxin (CDT) is the best characterized toxin of *Campylobacter spp*[7], one of the major virulence factors close to *Campylobacter spp*, pathogenesis in animals and humans is the cytolethal distending

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toxin (CDT) that results in diarrhea by interfering with the division and differentiation of cells in intestinal crypts[8,9]. The *cdt* gene cluster is encoded by the toxin action, built up of three adjacent genes: *cdtA, cdtB* and *cdtC* [10] All the three subunits are required needed for full toxin activity; cdtB encodes the active / toxic component of the toxin, while *cdtA and cdtC* are involved with binding to and internalization in to the host cell[11]. In *C. jejuni*, CDT causes progressive cellular distension within the eventual cell death[12]. The aim of this study was to investigating on cytolethal toxin from chicken in AL-Diwanyia city by using Real time PCR Technique.

MATERIALS AND METHODS

STOOL SAMPLES COLLECTION: 50 chicken stool samples were collected from different poultry field in Al-Diwanyia city. The samples were collected in 25ml sterile containers transported into laboratory and stored in a refrigerator until use for genomic DNA extraction.

GENOMIC DNA EXTRACTION

Bacterial genomic DNA was extracted from stool by using (AccuPrep® stool DNA Extraction Kit. Bioneer, Korea). 200mg stool sample was placed in 1.5ml microcentrifuge tube and 20µl 10mg/ml Proteinase K and 400ul stool lysis buffer was added and mixed by vortex, then incubated at 60C° for 10 minutes, then the tubes transferred in to centrifuged at 10000 rpm for 5 min, after that the supernatant was transferred in two new 1.5 ml microcentrifuge tube and genomic DNA extraction was done according to company instruction.

After that the extracted DNA was checked by Nanodrop spectrophotometer, then store in -20C° at refrigerator until perform PCR assay.

REAL-TIME PCR

Real-Time PCR technique was performed by using Syber green dye for detection and amplification of (cytolethal distending toxin gene) virulence factors gene in *Campylobacter jejuni*. The primes were designed in this study by using NCBI-Gen Bank recorded sequence for *Campylobacter jejuni* strain ATCC 29428 cytolethal distending toxin protein subunit A gene, complete cdt Gen Bank: (KC311548.1) and by using primer 3 plus design online. The primers were provided by (Bioneer Company, Korea) as show in the following table:

Table 1.

Primer		Sequence	Amplicon
CDT gene	F	AGCAAAGGATTTGGCGATGC	147bp
	R	TGCGTGATTGCTTGCATCAC	

The Real-Time PCR amplification reaction was done by using (AccuPowerTM 2X Green star qPCR master mix kit, Bioneer. Korea) and the qPCR master mix were prepared for each sample according to company instruction as following table:

Table 2.

qPCR master mix	Volume
Genomic DNA template	2.5µL
2X Green star master mix	25µL
CDT gene Forward primer (10pmol)	1µL
CDT gene Reverse primer (10pmol)	1µL
DEPC water	20.5µL
Total volume	50µL

These qPCR master mix reaction components that mentioned in table was placed in sterile white qPCR strip tubes and transferred in to Exispin vortex centrifuge for 3 minutes the place in MiniOpticon Real-Time PCR system and applied the following Thermocycler conditions as the following table:

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Table 3.

qPCR step	Temperature	Time	Repeat cycle
Initial Denaturation	95 C°	3 minute	1
Denaturation	95 C°	10 sec	45
Annealing\ Extension	60 C°	30 sec	
Detection(scan)			
Melting	60-95C°	0.5 sec	1

RESULTS

Real-Time PCR technique for detection of cytolethal distending toxin in *Campylobacter jejuni* were show in (41/ 50) at (82%) positive samples in chicken , by amplification of CDT gene in extracted DNA from feces samples as shown in the following figures :

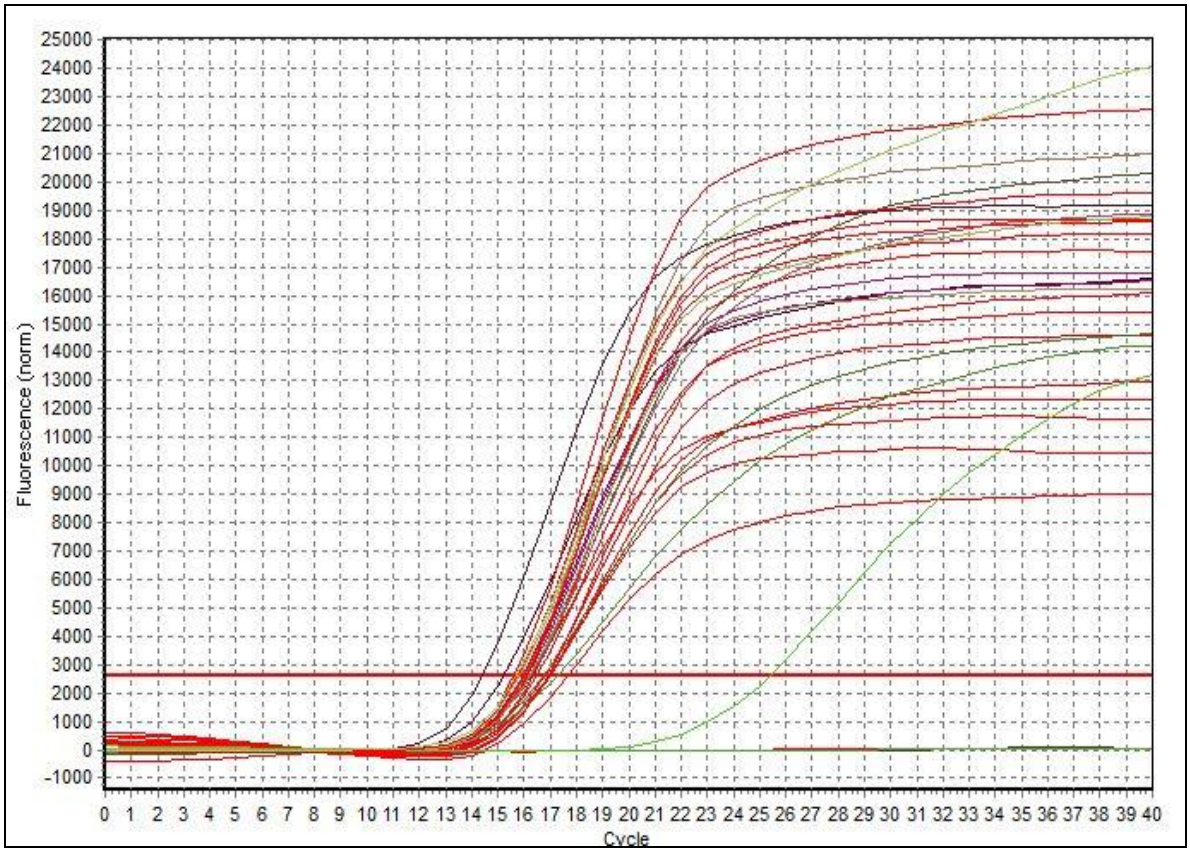

Figure 1. Real-Time PCR amplification plots for CDT gene that appeared at 13 to 23 cycle. The samples with amplification at 13 cycles contained very large amount of DNA while the samples with the amplification appeared at 28 cycles contained lower quantity of DNA for *Campylobacter jejuni*.

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+/- Assay SYBR

Pos	Name	+/- Result SYBR	Ct SYBR
A1	Campylobacter je	Positive	14.90
A2	Campylobacter je	Positive	15.70
A3	Campylobacter je	Positive	13.56
A4	Campylobacter je	Positive	15.43
A5	Campylobacter je	Positive	14.37
A6	Campylobacter je	Positive	13.88
B1	Campylobacter je	Positive	15.01
B2	Campylobacter je	Positive	15.23
B3	Campylobacter je	Positive	12.85
B4	Campylobacter je	Positive	15.06
B5	Campylobacter je	Positive	14.55
B6	Campylobacter je	Positive	13.57
C1	Campylobacter je	Positive	14.48
C2	Campylobacter je	Positive	15.28
C3	Campylobacter je	Negative	
C4	Campylobacter je	Positive	15.15
C5	Campylobacter je	Negative	
C6	Campylobacter je	Negative	
D1	Campylobacter je	Positive	14.88

Figure 2. Real-Time PCR endpoint analysis that display the positive and negative samples.

The specificity of CDT gene primers that amplification by Syber green based Real-Time PCR was determined by dissociation curve (Melt Curve). Where the positive amplification product samples show specific amplification at melt peak mainly at (Tm: 81C°) without primer dimer or nonspecific products. (Fig. 3)

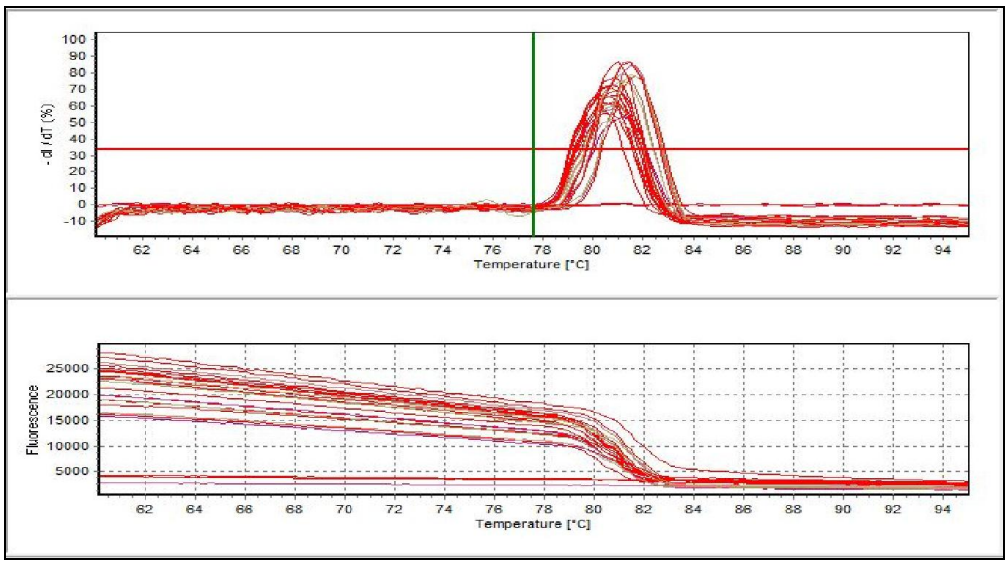

Figure 3.Real-Time PCR Melt curve that shows the melting point for CDT gene ranged from 80C° to 82C° for all samples, and the line from the highest peak to the button was detected that the melting point at 81C° slightly range above or low which represent the specific primers amplification.

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DISCUSSION

The prevalence of virulence factor cytolethal distending toxin (CDT) genes was investigated in isolates of *Campylobacter jejuni* originated from intestinal contents of broiler chicken, the cytolethal distending toxin (CDT) is one of the major virulence factors related to *Campylobacter jejuni* pathogenesis in humans and animals, it causes diarrhea by interfering with the division and differentiation of cells in intestinal crypts[8,9]. Other study by[13] who explains that occurrence of *Campylobacter spp*. strains carrying CDT genes in samples of poultry and vegetables are as an important vehicle for potentially virulent *Campylobacter spp*. The virulence factor gene cytolethal distending toxin (*cdt*) was investigated in isolates of *Campylobacter jejuni , C. coli , C. lanienae* and *C. lari* originated from intestinal contents and gall bladders of clinically healthy cattle.

High prevalence of cytolethal distending toxin (*cdt*) in the present study was agreement with many previous studies[14-15-16], Who found high prevalence of cytolethal distending toxin (*cdt*) and other virulence factors related to *Campylobacter jejuni* pathogenesis that causes diarrhea[16], indicated to the cdt genes of *C. jejuni* strains isolated from broiler carcasses and lettuce heads had been detected in 83.3% (20/24). In the present study, the percentage *of C. jejuni* that carried the CDT gene was similar to that reported in the international literature.

While a study in Poland[16] discovered that all three *cdt* genes were found in nearly all *C. jejuni* isolates from poultry carcasses chicken.

95% of three *cdt* genes were detected in the *C. jejuni* strains that have been secluded from poultry In Thailand[17], *C. jejuni* has been Identified in patients with diarrhea in Bangladesh, and the genes *cdtA , cdtB* and *cdtC* have been also found in 97.5% of the strains[18]. In this study Real-time PCR assay was establishment a rapid detection of *Campylobacter jejuni* that virulence factor cytolethal distending toxin (CDT) genes from stool. Specifity of detection by real time PCR assay was depended on the specific CDT gene that only amplification with *Campylobacter jejuni* nucleic acid. Other many study was used PCR technique in detection and analysis of Cytolethal distending toxin genes in *Campylobacter jejuni* and Campylobacter coli isolates[19]. There is no studies about *Campylobacter jejuni* in chicken in AL-Diwanyia city which used real-time PCR technique in detection CDT gene , therefore the present study aimed to used the most sensitive and specific real-time PCR in detection in *Campylobacter jejuni* that carried Cytolethal distending toxin gene.

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