# Carriage of Cdt-B Encoding *Campylobacter* spp., *Salmonella Enterica*, and *Yersinia Entercolitica* in Patients With Gastroenteritis and Irritable Bowel Syndrome

**Leila Ganji**
Health Reference Laboratory Reasearch Center, Reference Health Laboratory, Ministry of Health and Education

**Mohammad Hassan Shirazi**
Medical School of University of Tehran

**Masoud Alebouyeh** ( masoud.alebouyeh@gmail.com )
Shahid Beheshti University of Medical Sciences Pediatric Infections Research Center
https://orcid.org/0000-0001-7474-2515

**Parisa Eslami**
Department of Microbiology, Central Laboratory, Milad Hospital

**Mohammad Rahbar**
Health Reference Laboratory Research Center, Reference Laboratory, Ministry of Health and Eduaction

**Nasser Ebrahimi Daryani**
Tehran University of Medical Science

**Mohammad Reza Zali**
Shahid Beheshti University of Medical Sceince

**Research**

**Keywords:** Enteric pathogens, Irritable Bowel Syndrome, Gastroenteritis, Cytholethal distending toxin

**Posted Date:** September 18th, 2020

**DOI:** https://doi.org/10.21203/rs.3.rs-76649/v1

# Abstract

**Introduction**: Cytolethal distending toxin (Cdt) is one of the bacterial toxins that present in a variety of Gram-negative human pathogens, such as *E. coli, Salmonella* spp., and *Campylobacter* spp. CDT composed of three subunits encoded by three adjacent genes, including *cdtA*, *cdt*B and *cdt*C. It is approved that *cdtB* had toxic activity and caused DNA damage of the host cell. Despite its presence in different bacterial species, role of Cdt in acute and chronic infections, such as gastroenteritis and irritable bowel syndrome (IBS) is unclear. To analyze this correlation, we studied the prevalence of *cdt*B among different enteropathogenic bacteria in patients with gastroenteritis and IBS compared with healthy people.

**Materials and Methods**: In this cross-sectional descriptive study, 230 stool samples were collected from patients with gastroenteritis, IBS, and healthy people. The presence of Cdt-B encoding bacteria, including *Escherichia coli*, *Campylobacter* spp.*, Yersinia entercolitica*, *Providencia alkalifacience,* and *Salmonella enterica* was examined by polymerase chain reaction using genus specific primers.

**Results:** Out of 230 stool samples, Cdt-B encoding *Campylobacter* spp. were found in 34.6% (52/150), 6.25% (5/80), and 4% (2/50) of the patients with gastroenteritis, IBS, and the control group, respectively. Carriage of Cdt-B encoding *Salmonella enterica* was characterized among 5.3% (8/150) of the patients with gastroenteritis and 17.5% (14/80) of the IBS patients. Although none of the patients carried *cdtB* of *E. coli* and *Providencia* spp., *cdt*B of *Y. enterocolitica* was detected in 1 of the patients with gastroenteritis (0.6%). Statistical analysis showed significant correlation between infection with CdtB-encoding *Campylobacter* spp. and IBS-D subtype. No significant correlation was found between infection with Cdt-B encoding bacteria, and other clinical and demographic data.

**Conclusion:** Our results confirmed relatively higher frequency of Cdt-B encoding bacteria in the intestine of IBS patients and those with gastroenteritis compared with healthy individuals. Regarding the frequency of Cdt-B encoding *Salmonella* and *Campylobacter* bacteria, it was proposed that infection with these enteropathogens could be considered as a risk factor for the development or progression of IBS among the Iranian patients. Further studies are needed to establish this involvemet.


# Introduction

Cytholethal distending toxins (CDT) represent an emerging and unique toxin family. CDT is a heterotrimeric AB2 genotoxin, which consist of *cdt*A, *cdt*B and *cdt*C. CdtA and CdtC subunits bind to the host cell membrane whereas CdtB enter to the cell nucleolus and causes direct DNA damage due to DNase activite [1, 2]. Although CdtB is the most conserved subunit among all CDT-producing bacterial strains, its overall amino acid sequence showed diversity between 29 to 91% among different bacteria [3, 4].

CDT was first described in *Escherichia coli* by Johnson and Lior in the 1980s. *Gammaproteobacteria* and *Epsilonproteobacteria* are among main members of *Proteobacteria* that carry *cdt*, however, its presence in

*Firmicutes*, e.g. *Clostridioides difficile*, was also reported [4–6]. Within the *Epsilonproteobacteria*, CDT was found in the orders *Campylobacterialess* specially *Campylobacter* and *Helicobacter* species [7].

Many of these Gram-negative bacteria are considered as clinically important human pathogens that are responsible for gastroenteritis [8]. Involvement of these bacteria in development of chronic bowel disorders, such as irritable bowel syndrome (IBS), was reported in different studies [9, 10]. Accordingly, 3–30% of people with IBS experience their symptoms after an episode of acute gastroenteritis (Post-infectious IBS, PI-IBS) [11].

Currently, no definite virulence factors are characterized in these bacteria in association to PI-IBS. Dysbiosis of the gut microbiota and alteration of microbial population in this organ could accelerate growth of more virulent bacteria, which promote functional disorders through their interaction with the host [12–14]. This interaction and the functional disorder, including chronic abdominal pain and alerted intestinal habits, could occur by unknown bacterial virulence factors [15, 16]. CDT of *Campylobacter* spp. is the only proposed virulence factors that elevated antibody titers against one of its subunits (CdtB), were shown in diarrhea-dominant form of IBS [17, 18]. This involvement may cause through cross-reaction of the antibodies with vinculin in the host gut [19–21]. Despite wide distribution of this family of toxins in members of the enteric bacteria, little is known about the prevalence of Cdt-B encoding bacteria and their association with distinct types of IBS (IBS with diarrhea, IBS with constipation, and mixed-types) promotion or exacerbation of the disease. This study was aimed to assess the presence of *cdt*B gene among different enteric bacteria including *Y. entercolitica, S. enterica, E. coli, Providencia alcalifaciens*, *Aggregatibacter actinomycetemcomitans*, and *Campylobacter* spp. among patients with IBS and gastroenteritis in compare to healthy people.

# Methods And Materials

### Samples collection:

This cross-sectional descriptive study was conducted on 230 stool samples that obtained from patients with acute gastroenteritis and IBS. A control group of healthy volunteers (50 samples) was also included in the study. Fresh stool samples were collected in clean containers and the samples were immediately transferred to the laboratory under cold chain. Adult patients with functional bowel disorders were interviewed by experienced physicians and fulfilled a questionnaire that was designed according to Rome IIi criteria for IBS [22]. According symptoms, they was classified as either diarrhea-predominant (IBS-D), constipation-predominant (IBS-C), or with alternating stool pattern (mixed IBS). Exclusion criteria were included, intestinal disturbance (Celiac disease and lactose intolerance), recent history of hospitalization (>24 h), antibiotic prescription within the last 3 months, surgery of the gastrointestinal tract, local and systematic inflammatory diseases, defined diet, food allergy, and pregnancy. Healthy controls were selected from people of the same age, who enrolled in routine medical check-ups in the hospital. These people reported no history of the gastrointestinal disorders and the criteria described above.

Isolation and characterization of the culturable bacteria was performed using selective and specific culture media as before [14]. Briefly, a total of 100 µl volume of the suspension was cultured on different culture media, including MacConky agar (Merck, Germany) as a selective medium for members of Enterobacteriacea, Brucella agar supplemented with sheep blood (5%), Campylobacter supplement for detection of *Campylobacter* spp., and Clostridium selective agar and Mannitol salt agar for isolation of Clostridia and *Staphylococci*, respectively. The growth of aerobic and anaerobic bacteria was analyzed at 37$^{O}$C after 24- 48 h incubation under aerobic and anaerobic conditions (Anoxomat: MART Microbiology B.V. 0% O2, 10% H2, 10% CO2 and 80% N2). Suspicious colonies were identified by routine microbiological and biochemical tests [14].

**DNA preparation**:

Total DNA of the samples was extracted using DNA Stool Kit (Bionner, South Korea) according to the manufacturer's instructions. The concentration of DNA was measured by Nanodrop (Eppendorf-Germany). All DNA extracts were stored at −20 °C until use.

**Identification of *cdt*B by PCR**:

In this study specific primers were designed for characterization of *cdtB* in *Y.entercolitica, S. enterica, E. coli, Providencia alcalifaciens*, and *Aggregatibacter actinomycetemcomitans* (Table 1). Accoringly, homology of *cdt*B were determined using CLC Sequence Viewer v.6.0, and appropriate regions were selected. Amplification of the *cdtB* were carried out in mastermix volume of 25 µl containing 5 *µl* of DNA template, 0.5 mM of each dATP, dGTP, dCTP and dTTP (gene fanavaran, Iran), PCR (10x) buffer (Gene fanavaran, Iran), 0.3 *µl* (10 pmol) of each forward and reverse primer, 1x Tag DNA polymerase buffer (Gene fanavaran, Iran), and 0.2 *µl* (5 U/ *µl*) of Taq DNA polymerase (Fermentase, Germany). Amplified products were visualized in agarose gels (1.5%) in TAE buffer along with a mixed DNA ladder which stained with ethidium bromide.

**Sequencing of PCR products.**

PCR products of each suspected sample were purified using QIA Quick Spin Column (Qiagene, Germany). Then, PCR products were automatically sequenced in Sanger sequencing service. Resulted sequences were aligned and analyzed using Blast, Chromas, and BioEdit software.

**Statistical analysis.**

Statistical analysis was performed with SPSS software (Version 23, Co Ltd, Tokyo, Japan). Data were expressed as mean ± standard deviation for continuous and frequency percentage for nominal and categorical variables. The comparison of qualitative variables between groups analyzed by the Pearson Chi- square test. The results were considered to be significant if *p*-values were ≤0.05.

# Results

## Clinical information of patients and controls.

Out of 80 patients with IBS, 18 had IBS-D (mean age, 37.55 ± 3.7 y), 29 had IBS-C (mean age, 37.26 ± 2.8 y), and 33 had IBS-M (mean age, 42.68 ± 2.1 y). In addition, 150 patients with gastroenteritis (mean age, 41.3 ± 2.1 y), and 50 healthy controls (mean age, 37.9 ± 2.1 y) were recruited into the study.

The results showed that the prevalence of IBS was significantly higher in women than in men (56/24) ($p$ <0.05). The commonest subtype of IBS in female patients was IBS-C (86.2%); while, IBS-M was the most frequent type among male (33.33%). Diarrhea-predominant IBC was the same in men and female. Anxiety was significantly higher among women in compare with men ($P$ < 0.05). Although, several symptoms including abdominal bloating, cramp, and stress were more common in female than men. However, this difference was not significant (Table 2).

The most common symptom associated with constipation in subjects with IBS-C was abdominal bloating (89.65 %) followed by bellyache (75 %). While, IBS-D subjects felt a higher degree of abdominal cramp in their daily lives than two other subtypes ($P$= 0.01). Additionally, there was a significant correlation between IBS-C and IBS-M with anxiety. Interestingly, the anxiety degree was significantly associated with bloating ($P$= 0.02) and abdominal pain ($P$<0.01). With regard to the IBS patient subgroups, there was no statistically difference between age and subtype of IBS.

## Distribution of *cdt*B gene variants in the fecal DNA extracts.

The frequency of *cdt*B varied between patients with IBS, gastroenteritis, and healthy people. *cdt*B of *Campylobacter* showed high- frequency in stool samples of gastroenteritis patients (34.6%, 52/150); which was higher than those characterized in patients with IBS and healthy people (6.25%, 5/80 and 4%, 2/50, respectively); the difference was statistically significant ($p$ <0.05). Significant association also were seen between *cdt*B of *Campylobacter* spp. and IBS-D patients. *cdt*B of *S. enterica* was detected in 8 (5.3%) and 14 (17.5%) of the patients with gastroenteritis and IBS, respectively. There was a significant difference between the presence of *cdt*B of *S. enterica* in patients with IBS and gastroenteritis compared with healthy subjects ($p$ <0.05). However, the presence of *cdt*B of *S. enterica* didn't show a correlation with IBS types (24.24%, 13.79 %, and 11.11% in IBS-M, IBS-C, and IBS-D patients, respectively). *cdt*B of *E. coli* and *P. alkalifacience* was not detected in any of the patients with IBS, gastroenteritis, and in the control group. The results also indicated that only 0.6% of the patients with gastroenteritis carried *cdt*B of *Y.entercolitica* (Table 3). Statistical analyses showed no significant correlation between Cdt-B encoding bacteria and gender or other demographic data among the studied patients with IBS.

## Sequence accession number

Nucleotide sequence analysis of all amplified PCR products showed 100% identity to *cdtB* of *Campylobacter spp., S.enterica* and *Y.entercolitica.* The accession numbers of the sequences of the PCR products submitted to GenBank were KT008107.1, KR778819.1, KT008106.1 (https://www.ncbi.nlm.nih.gov).

# Discussion

The overall objectives of the current study were to determine the distribution of *cdt*B gene among different enteropathogenic bacteria in subjects with IBS and gastroenteritis and its possible role in disease.

According to our results, the prevalence of *cdt*B of *Campylobacter* spp. was 6.25% in patients with IBS and 34.6% in patients with gastroenteritis in comparison with healthy people (3.75%). Similar to our results, the high prevalence of the *cdtB* gene was detected among *Campylobacter* isolates, in gastroenteritis patients [24]. Peter KE *et al* were determined the *cdtB* gene among 67% of *C. jejuni* and 19% of *C. coli* isolates recovered from cases of gastroenteritis. Indeed, distribution of *Campylobacter* encoding *cdt*B was detected in 5% of patients with IBS by Burliaeva *et al* [25]. We compared the clinical data of the IBS patiats with Cdt-B encoding bacteria. The comparison revealed that the frequency of Cdt-B encoding *Campylobacter* spp. was significantly higher among IBS-D patients (16.66%) than other. Burliaeva *et al* showed that wild-type *C. jejuni* strain, but not its Cdt-lacking isogenic mutant of *cdt*B was able to induce chronic altered bowel patterns and mild chronic rectal inflammation [25, 26]. Indeed, anti-CdtB antibodies has been proposed as a apecific biomarker for diarrhea-predominant irritable bowel syndrome [17, 18]. It seems that CDT has the ability to attack the cells of intestinal villi, and cause disease [26, 27]. Whether *Campylobacter* strains that produce *cdtB* exclusively are associated with diarrhea in patients with irritable bowel syndrome needs more investigation. In this study, no significant association was seen between the *Campylobacter* encoding *cdt*B and other clinical symptome including abdominal bloating. No significant correlation also was found between Cdt-B encoding *Campylobacter* spp. and gender among IBS patients, while most of IBS patients were female. According our knowledge, studies on this subject are limited.

This study showed that 14% of patients with IBS and 5.3% of patients with gastroenteritis were positive for *cdt*B of *S. enterica*. High distribution of *cdt*B gene was unpredictable. Although *cdt*B was not detected in the control group. Among IBS patients who had *cdt*B positive *S.enterica*, 11 (13.75%), 9 (11.25%), and 5(6.25%) had bloating, abdominal pain, and abdominal cram, respectively. Precence of *cdt*B were reported in typhoidal and nontyphoidal *S. enterica* serovars [5, 28–31]. *Mezal et al* showed carriage of *cdt*B gene among all isolates of *Salmonella enterica* serovar *Javiana* in patients with gastrointestinal [5]. An increased level of invasiveness for these isolates was shown in HeLa cell cultures, which mediated by CDT. Possible involvement of CDT of *Salmonella* in the development or progression of IBS could be also supported by previous results that described association of *Salmonellosis* and IBS [5].

The data obtained showed that only one Cdt-B encoding *Y. entercolitica* was detected in gastroenteritis patients. However, recent evidence demonstrated that this pathogen is linked with chronic health sequelae, including functional gastrointestinal disorders (FGD) such as Inflammatory bowel disease (IBD) [32].

According to the results, none of the samples (IBS, gastroenteritis and healthy people) were positive for *cdt*B of *E.coli*. Indeed, all *E.coli* which isolated from paints with IBS, gastroenteritis and healthy people

were investigated for *cdt*B by PCR. Meza-Segura *et al*, determined the *E.coli* strains harboring *cdt* genes among 1306 young children with acute diarrhea. Thirteen patients (1%) had *E. coli* strains harbouring *cdt* genes; only five of them were negative for all other tested pathogens [33]. These finding suggest that CDT-producing *E. coli* strains are an infrequent, albeit significant, cause of severe diarrheal illness in children. In another study, *cdt*B gene weres found in 1.4% of 366 *E. coli* strains isolated from stool specimens of patients with acute diarrhea in Calcutta, India [34].

In our study, we cannot find the *P.alcalifacience* CdtB and AacCDT among our samples. However, Shiam *et al* could detected *P.alcalifacience* harboring CdtB from patients with diarrhea [35]. For the first time, they reported that certain clinical *P. alcalifaciens* strains could produce variants of the CDTs compared [35].

# Conclusion

Our data showed high distribution of *cdt*B gene among *Campylobacter* spp. and *Salmonella* spp. in stool samples of patients with IBS and gastroenteritis. These findings proposed that infection with CdtB encoding bacteria could be a risk factor for development of IBS. In addition, significant association was seen between CdtB encoding *Campylobacter* spp. and diarrhea-predominant irritable bowel syndrome. No significant correlation was found between CdtB encoding *Campylobacter* spp. and *S. enterica* and gender among the IBS patients. Further studies are needed to establish this correlation.

# Declarations


### Acknowledgment

Many thanks go to Prof. Saeid Bouzari (Pasture Institute of Iran, *E. coli* Reference Laboratory, Iran) for providing DNA of *Cdt*B encoding *E.coli*. The study was approved by ethical committee of Department of Pathobiology, Tehran university of Medical Sciences, Tehran, Iran (TUMS. ethics.code 92-02-27-22726).


# References


1. Lara-Tejero Ma, Galán JE: **CdtA, CdtB, and CdtC form a tripartite complex that is required for cytolethal distending toxin activity**. *Infection and immunity* 2001, **69**(7):4358-4365.
2. Pickett CL, Whitehouse CA: **The cytolethal distending toxin family**. *Trends in microbiology* 1999, **7**(7):292-297.
3. Elwell CA, Dreyfus LA: **DNase I homologous residues in CdtB are critical for cytolethal distending toxin-mediated cell cycle arrest**. *Molecular microbiology* 2000, **37**(4):952-963.
4. Jinadasa RN, Bloom SE, Weiss RS, Duhamel GE: **Cytolethal distending toxin: a conserved bacterial genotoxin that blocks cell cycle progression, leading to apoptosis of a broad range of mammalian cell lineages**. *Microbiology* 2011, **157**(7):1851-1875.

5. Mezal EH, Bae D, Khan AA: **Detection and functionality of the CdtB, PltA, and PltB from Salmonella enterica serovar Javiana**. *Pathogens and disease* 2014, **72**(2):95-103.
6. Osek J: **Detection of cytolethal distending toxin genes in Shiga toxin-producing Escherichia coli isolated from different sources**. *Bull Vet Inst Pulawy* 2005, **49**:153-156.
7. Whitehouse CA, Balbo PB, Pesci EC, Cottle DL, Mirabito PM, Pickett CL: **Campylobacter jejuni cytolethal distending toxin causes a G2-phase cell cycle block**. *Infection and immunity* 1998, **66**(5):1934-1940.
8. Humphries RM, Linscott AJ: **Laboratory diagnosis of bacterial gastroenteritis**. *Clinical microbiology reviews* 2015, **28**(1):3-31.
9. Schwille-Kiuntke J, Enck P, Zendler C, Krieg M, Polster A, Klosterhalfen S, Autenrieth I, Zipfel S, Frick JS: **Postinfectious irritable bowel syndrome: follow-up of a patient cohort of confirmed cases of bacterial infection with Salmonella or Campylobacter**. *Neurogastroenterology & Motility* 2011, **23**(11).
10. Marshall JK, Thabane M, Borgaonkar MR, James C: **Postinfectious irritable bowel syndrome after a food-borne outbreak of acute gastroenteritis attributed to a viral pathogen**. *Clinical Gastroenterology and Hepatology* 2007, **5**(4):457-460.
11. Thornley JP, Jenkins D, Neal K, Wright T, Brough J, Spiller RC: **Relationship of Campylobacter toxigenicity in vitro to the development of postinfectious irritable bowel syndrome**. *The Journal of infectious diseases* 2001, **184**(5):606-609.
12. Shukla R, Ghoshal U, Dhole TN, Ghoshal UC: **Fecal microbiota in patients with irritable bowel syndrome compared with healthy controls using real-time polymerase chain reaction: an evidence of dysbiosis**. *Digestive diseases and sciences* 2015, **60**(10):2953-2962.
13. Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ: **Dysbiosis of the gut microbiota in disease**. *Microbial ecology in health and disease* 2015, **26**(1):26191.
14. Ganji L, Alebouyeh M, Shirazi MH, Eshraghi SS, Mirshafiey A, Daryani NE, Zali MR: **Dysbiosis of fecal microbiota and high frequency of Citrobacter, Klebsiella spp., and Actinomycetes in patients with irritable bowel syndrome and gastroenteritis**. *Gastroenterology and hepatology from bed to bench* 2016, **9**(4):325.
15. Chassard C, Dapoigny M, Scott KP, Crouzet L, Del'homme C, Marquet P, Martin JC, Pickering G, Ardid D, Eschalier A: **Functional dysbiosis within the gut microbiota of patients with constipated-irritable bowel syndrome**. *Alimentary pharmacology & therapeutics* 2012, **35**(7):828-838.
16. Chong PP, Chin VK, Looi CY, Wong WF, Madhavan P, Yong VC: **The Microbiome and Irritable Bowel Syndrome–A Review on the Pathophysiology, Current Research and Future Therapy**. *Frontiers in microbiology* 2019, **10**:1136.
17. Pimentel M, Morales W, Rezaie A, Marsh E, Lembo A, Mirocha J, Leffler DA, Marsh Z, Weitsman S, Chua KS: **Development and validation of a biomarker for diarrhea-predominant irritable bowel syndrome in human subjects**. *PLoS One* 2015, **10**(5).

18. Buff E: **A diagnostic blood test for irritable bowel syndrome: a utopia?***Revue medicale suisse* 2015, **11**(484):1628.
19. Pimentel M, Morales W, Pokkunuri V, Brikos C, Kim SM, Kim SE, Triantafyllou K, Weitsman S, Marsh Z, Marsh E: **Autoimmunity links vinculin to the pathophysiology of chronic functional bowel changes following Campylobacter jejuni infection in a rat model**. *Digestive diseases and sciences* 2015, **60**(5):1195-1205.
20. Schmulson M, Balbuena R, de Law CC: **Clinical experience with the use of anti-CdtB and anti-vinculin antibodies in patients with diarrhea in Mexico**. *Revista de Gastroenterología de México (English Edition)* 2016, **81**(4):236-239.
21. Pimentel M, Chang C: **Methods of comparing anti-vinculin and anti-cytolethal distending toxin antibodies as they relate to irritable bowel syndrome**. In.: Google Patents; 2017.
22. Whitehead WE, Drossman DA: **Validation of symptom-based diagnostic criteria for irritable bowel syndrome: a critical review**. *The American journal of gastroenterology* 2010, **105**(4).
23. Ceelen LM, Decostere A, Ducatelle R, Haesebrouck F: **Cytolethal distending toxin generates cell death by inducing a bottleneck in the cell cycle**. *Microbiological research* 2006, **161**(2):109-120.
24. Peters KE, Chang Y-C, Duhamel GE, Sultan AA, Doiphode S, Ibrahim EM, Mohammed HO: **Cytolethal Distending Toxin in Salmonella and Campylobacter spp. Isolated from Food Animals and Gastroenteritis Cases in Qatar**. *Global Journal of Health Science* 2017, **9**(10).
25. Burliaeva E, Bulakhov A, Pilipenko V, Isakov V, Sheveleva S: **Campylobacter in stool of patients with irritable bowel syndrome**. *Eksperimental'naia i klinicheskaia gastroenterologiia= Experimental & clinical gastroenterology* 2010(11):53-57.
26. Pokkunuri V, Pimentel M, Morales W, Jee S-R, Alpern J, Weitsman S, Marsh Z, Low K, Hwang L, Khoshini R: **Role of cytolethal distending toxin in altered stool form and bowel phenotypes in a rat model of post-infectious irritable bowel syndrome**. *Journal of neurogastroenterology and motility* 2012, **18**(4):434.
27. Scuron MD, Boesze-Battaglia K, Dlakić M, Shenker BJ: **The cytolethal distending toxin contributes to microbial virulence and disease pathogenesis by acting as a tri-perditious toxin**. *Frontiers in cellular and infection microbiology* 2016, **6**:168.
28. Miller R, Wiedmann M: **Dynamic Duo—The Salmonella Cytolethal Distending Toxin Combines ADP-Ribosyltransferase and Nuclease Activities in a Novel Form of the Cytolethal Distending Toxin**. *Toxins* 2016, **8**(5):121.
29. Haghjoo E, Galán JE: **Salmonella typhiencodes a functional cytolethal distending toxin that is delivered into host cells by a bacterial-internalization pathway**. *Proceedings of the National Academy of Sciences* 2004, **101**(13):4614-4619.
30. Rodriguez-Rivera LD, Bowen BM, den Bakker HC, Duhamel GE, Wiedmann M: **Characterization of the cytolethal distending toxin (typhoid toxin) in non-typhoidal Salmonella serovars**. *Gut pathogens* 2015, **7**(1):19.

31. Williams K, Gokulan K, Shelman D, Akiyama T, Khan A, Khare S: **Cytotoxic mechanism of cytolethal distending toxin in nontyphoidal Salmonella serovar (Salmonella Javiana) during macrophage infection**. *DNA and cell biology* 2015, **34**(2):113-124.
32. Porter CK, Choi D, Cash B, Pimentel M, Murray J, May L, Riddle MS: **Pathogen-specific risk of chronic gastrointestinal disorders following bacterial causes of foodborne illness**. *BMC gastroenterology* 2013, **13**(1):46.
33. Meza-Segura M, Zaidi MB, Maldonado-Puga S, Huerta-Cantillo J, Chavez-Dueñas L, Navarro-Garcia F, Estrada-Garcia T: **Cytolethal distending toxin-producing Escherichia coli strains causing severe diarrhoea in young Mexican children**. *JMM case reports* 2017, **4**(2).
34. Pandey M, Khan A, Das SC, Sarkar B, Kahali S, Chakraborty S, Chattopadhyay S, Yamasaki S, Takeda Y, Nair GB: **Association of cytolethal distending toxin locus cdtB with enteropathogenic Escherichia coli isolated from patients with acute diarrhea in Calcutta, India**. *Journal of clinical microbiology* 2003, **41**(11):5277-5281.
35. Shima A, Hinenoya A, Asakura M, Sugimoto N, Tsukamoto T, Ito H, Nagita A, Faruque SM, Yamasaki S: **Molecular characterizations of cytolethal distending toxin produced by Providencia alcalifaciens strains isolated from patients with diarrhea**. *Infection and immunity* 2012, **80**(4):1323-1332.

# Tables

Table 1
Primers that were used in this study.

| Bacteria | Sequence )5´-3´( | PCR product (bp) | Reference |
|---|---|---|---|
| *Yersinia entercolitica* | TAGCAATAGCAAATGGATAG<br>ATCTGCTCTAATTCTTGA | 376 | This study |
| *Salmonella enterica* | TTCTGACCATGATCATCTG<br>AGATTCCAGGTGTATTCATC | 283 | This study |
| *Escherichia coli* | AGGCCATTAACTGGATGATT<br>TTTCCWRCTACHGCATAATC | 178 | This study |
| *Campylobacter jejuni* | GTTGGCACTTGGAATTTGCAAGGC<br>RTTRAARTCNCCYAADATCATCC | 470 | Bang 2003 |
| *Providencia alkalifacience* | GTAGCGAGCTTGGAATTTGCAAGGC<br>TTTRAATTTCAGAAAGATCATCC | 680 | This study |

Table 2
Frequency of symptoms in patients with IBS and gastroenteritis.

| Type of symptoms | IBS-C | IBS-D | IBS-M |
|---|---|---|---|
| | N (%) | N (%) | N (%) |
| Total number | 29/80 (14.5%) | 18/80 (22.5%) | 33/80 (15.4%) |
| Anxiety | 21 (72.41%) | 8 (44.44%) | 17(51.51%) |
| Yes | 8 (27.58%) | 10 (55.55%) | 16 (48.48%) |
| No | | | |
| Abdominal pain | 21 (72.41%) | 14 (77.77%) | 27 (81.81%) |
| Yes | 8 (27.58%) | 4 (22.22%) | 6 (18.18%) |
| No | | | |
| Abdominal Cramp | 9 (31.03%) | 15(83.33%) | 17 (51.51%) |
| Yes | 20 (68.96%) | 3 (16.66%) | 16 (48.48%) |
| No | | | |
| Abdominal bloating | 26 (89.65%) | 3 (16.66%) | 30 (90.9%) |
| Yes | 3(10.34%) | 15(83.33%) | 3(9.09%) |
| No | | | |
| Stress | 22 (75.86%) | 12(66.66%) | 20 (60.6%) |
| Yes | 7 (24.13%) | 6 (33.33%) | 13 (39.39%) |
| No | | | |
| Infection with CdtB encoding bacteria | 4 (13.79%) | 2 (11.11%) | 8 (24.24%) |
| *Salmonella* | 1 (3.44%) | 3 (16.66%) | 1 (3.03%) |
| *Campylobacter* | | | |
| Others | | | |

Table 3
Presence of *cdt*B in enteric pathogenic bacteria among patients with Irritable bowel syndrome and gastroenteritis.

| | *P. alkalifaciance* | *Y. entercolitica* | *E. coli* | *S. enterica* | *Campylobacter s*pp. |
|---|---|---|---|---|---|
| IBS (n = 80) | 0 | 0 | 0 | 14 (17.5%) | 5 (6.25%) |
| Gastroenteritis (n = 150) | 0 | 1(0.6%) | 0 | 8 (5.3%) | 52 (34.6%) |
| Control (n = 50) | 0 | 0 | 0 | 0 | 3 (3.75%) |