

## Cytolethal Distending Toxin (CDT)-Negative *Campylobacter jejuni* Strains and Anti-CDT Neutralizing Antibodies Are Induced during Human Infection but Not during Colonization in Chickens

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The cytolethal distending toxin (CDT) of *Campylobacter jejuni* was detectable, using an *in vitro* assay, in most but not all of 24 strains tested. The reason for the absence of toxin activity in these naturally occurring CDT-negative *C. jejuni* strains was then investigated at the genetic level. CDT is encoded by three highly conserved genes, *cdtA*, *-B*, and *-C*. In the CDT-negative strains, two types of mutation were identified. The CDT activities of *C. jejuni* strains possessing both types of mutation were successfully complemented with the functional genes of *C. jejuni* 11168. The first type of mutation comprised a 667-bp deletion across *cdtA* and *cdtB* and considerable degeneration in the remainder of the *cdt* locus. Using a PCR technique to screen for this deletion, this mutation occurred in fewer than 3% of 147 human, veterinary, and environmental strains tested. The second type of mutation involved at least four nonsynonymous nucleotide changes, but only the replacement of proline with serine at CdtB position 95 was considered important for CDT activity. This was confirmed by site-directed mutagenesis. This type of mutation also occurred in fewer than 3% of strains as determined using a LightCycler biprobe assay. The detection of two CDT-negative clinical isolates raised questions about the role of CDT in some cases of human campylobacteriosis. To determine if anti-CDT antibodies are produced in human infection, a toxin neutralization assay was developed and validated using rabbit antisera. Pooled human sera from infected patients neutralized the toxin, indicating expression and immunogenicity during infection. However, no neutralizing antibodies were detected in colonized chickens despite the expression of CDT in the avian gut as indicated by reverse transcription-PCR.

*Campylobacter jejuni* and *Campylobacter coli* are major causes of acute human bacterial enteritis in industrialized countries (35). These *Campylobacter* species asymptotically colonize the intestinal tracts of most mammals and birds (24), and one major route of human campylobacteriosis is assumed to be the consumption of contaminated poultry meat products (10). The pathogenic mechanisms by which campylobacters cause diarrhea are as yet unknown, although motility, adhesion, and invasion have been implicated (38). Several toxic activities have been reported, but their roles in disease remain debatable (37).

The best-characterized *Campylobacter* toxin is the cytolethal distending toxin (CDT). CDT production has been described in several gram-negative bacteria, including *Escherichia coli* (30, 33), *Haemophilus ducreyi* (7), *Actinobacillus actinomyces-temcomitans* (21), *Shigella dysenteriae* (25, 26), and *Helicobacter* spp. (42). However, not all of these species are implicated in enteric disease. In *C. jejuni*, CDT causes progressive cellular distension with eventual cell death (16). These morphological changes appear to be a consequence of alterations in the pro-

gression of the cell cycle, in particular cell cycle arrest in the G<sub>2</sub>/M phase (6, 8, 28, 41).

CDT production is dependent on the expression of three tandem genes, *cdtA*, *cdtB*, and *cdtC* (31). The CdtA, CdtB, and CdtC proteins form a tripartite holotoxin complex required for CDT activity (18). Current evidence indicates that *cdtB* encodes the active/toxic component of the toxin, while *cdtA* and *cdtC* are involved with binding to and internalization into the host cell (18, 19).

The role of CDT in human campylobacteriosis is unclear. Although, all *C. jejuni* strains tested to date appear to possess the *cdt* genes (11, 12, 31), the levels of toxin activities expressed are strain dependent, with two strains (~1.2%) reported to produce no detectable levels of CDT *in vitro* (2, 12). The explanation for such CDT-negative strains is currently unknown. In this study we have investigated the molecular basis of this using eight *C. jejuni* CDT-negative strains isolated from human diarrheic stools ( $n = 2$ ), bacteremia (blood) ( $n = 2$ ), a sheep ( $n = 1$ ), a poultry processing plant ( $n = 1$ ), and a broiler ( $n = 2$ ). The results indicated that lack of the CDT phenotype was a consequence of either major deletions (51 and 667 bp) in or around *cdtB* or one or more point mutations within the *cdtABC* genes. Site-directed mutagenesis and complementation were used to confirm these observations. A PCR assay and a LightCycler BiProbe assay were developed to screen 123 randomly selected veterinary and human *Campylobacter* iso-

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