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SHORT COMMUNICATION

Detection of *cdtA*, *cdtB*, and *cdtC* genes in *Campylobacter jejuni* by multiplex PCR

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Abstract

A multiplex PCR was developed for simultaneous detection of the cytolethal distending toxin (*cdt*) genes of *Campylobacter jejuni*. Three primer pairs targeting each one of the *cdtA*, *cdtB* and *cdtC* genes were designed and combined in the same PCR reaction. The assay was evaluated with 100 *C. jejuni* strains recovered from humans and animals and it was found to be rapid and specific. Two isolates presented several deletions affecting both *cdtA* and *cdtB* genes. High prevalence (98%) of the three *cdt* genes was found among isolates of different geographic origins.

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Keywords: *cdtA*; *cdtB*; *cdtC*; *Campylobacter jejuni*; Multiplex PCR

Introduction

The food-borne pathogen *Campylobacter* is a leading cause of gastrointestinal human infections in many industrialized countries, and particularly *Campylobacter jejuni* is the most common species implicated (Friedman et al., 2000). Several virulence factors have been reported to explain the pathogenesis of *Campylobacter* infections in human (Bacon et al., 2002; Fry et al., 2000; Konkel et al., 2004; Lara-Tejero and Galan, 2000; Bereswill and Kist, 2003), among them a cytolethal-distending toxin (CDT) that affects the epithelial cell layer and causes progressive cellular distension and death in several cell lines (Johnson

and Lior, 1988; Pickett et al., 1996). The CDT activity is encoded by the *cdt* genes, which in the case of *C. jejuni* consist of three adjacent or slightly overlapping genes named *cdtA*, *cdtB* and *cdtC* (Pickett et al., 1996).

Recently, a number of studies aimed at detecting the *cdt* genes among *C. jejuni* isolates from different sources has been reported (Bang et al., 2003a, b). However, the prevalence of these genes among *Campylobacter* isolates from reservoirs and potential sources of infection has not been investigated so far. Studies on these topics are still limited but they are needed to determine if all *Campylobacter* isolates are equally virulent to humans (Bang et al., 2003b). Current identification of the *cdt* genes is based on simplex PCR assays. In this work, we have developed and optimized a multiplex PCR to allow the simultaneous detection of the *cdtA*, *cdtB* and *cdtC* genes from *C. jejuni*.

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Materials and methods

Bacterial strains and culture media

A total of 100 *C. jejuni* isolates derived from humans and animals were used. *C. jejuni* strains, isolation sources and countries are recorded in Table 1. *C. jejuni* NCTC 11168 was used as reference strain in this study. The isolates were grown on Mueller-Hinton agar (Oxoid) supplemented with 5% of laked horse blood (Oxoid) and incubated at 42 °C in a microaerobic atmosphere containing 5% O₂, 10% CO₂ and 85% N₂.

PCR primer design

Oligonucleotide primers were selected from published sequences (GenBank, accession number [NC_002163](#)) using Primer 3 software. For *cdtA*, a 422-bp fragment was amplified by using primers CDTA-F (5'-TACTCCTATTACCCACC-3') and CDTA-R (5'-AATTTGAACCGCTGTATTGCTC-3'). In the case of *cdtB*, a 531-bp product was obtained by using primers CDTB-F, 5'-AGGAACCTTACCAAGAA-CAGCC-3', and CDTB-R, 5'-GGTGGAGTATAGG-TTTGTTGTC-3'. To amplify a *cdtC* region of 339 bp, the primers were CDTC-F, 5'-ACTCCTACTGGA-GATTTGAAAG-3', and CDTC-R, 5'-CACAGCTGA-AGTTGTTGTTGGC-3'. We also used primer pairs

that amplified *Campylobacter* 23S rRNA sequences (Wang et al., 2002) and the entire *cdtABC* cluster of *C. jejuni* when required (Bang et al., 2003a).

Multiplex PCR conditions

Genomic DNA was extracted and purified from overnight cultures by using the PrepMan™ Ultra kit (Applied Biosystem). Multiplex PCR was carried out in 25 µl reaction volumes containing 1 × PCR reaction buffer (100 mM Tris-HCl, 500 mM KCl, pH 8.3), 3 mM MgCl₂, 0.2 µM of each primer, 200 µM deoxynucleotides, 2 U Taq polymerase (Applied Biosystem), and 80 ng genomic DNA. PCRs were performed on a Robocycler 96 thermal-cycler (Stratagene), with an initial denaturation step of 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 57 °C for 1 min, and 72 °C for 1 min, and a final extension step at 72 °C for 5 min. PCR products were visualized by electrophoresis in a 2% agarose gel.

Nucleotide sequencing

PCR products were purified using a QIAquick PCR purification kit (Qiagen), and sequenced by Sistemas Genómicos SA (Valencia, Spain). The GenBank accession numbers of the deposited sequences are [AY957458](#) (strain CNET-025), and [AY957459](#) (strain CNET-083).

Table 1. *Campylobacter jejuni* strains used in this study

Source	Name	Geographic origin
Human (<i>n</i> = 76)	CH-001 to CH-064; CC-68 to CC-76	Spain
	CNET-002; CNET-004; CNET-047	Denmark
	CNET-005; CNET-007	Scotland
	CNET-009; CNET-010; CNET-012	Northern Ireland
	CNET-031	The Netherlands
	CNET-033; CNET-035; CNET-036; CNET-099; CNET-100	United Kingdom
	CNET-039; CNET-040	Belgium
Flock (<i>n</i> = 5)	CNET-016; CNET-017; CNET-018	The Netherlands
	CNET-022; CNET-023	Denmark
Wild birds (<i>n</i> = 2)	CNET-025; CNET-026	Finland
Cattle (<i>n</i> = 5)	CNET-052	The Netherlands
	CNET-055	Northern Ireland
	CNET-056; CNET-057	Denmark
	CNET-058	United Kingdom
Poultry (<i>n</i> = 6)	CNET-065	Denmark
	CNET-083; CNET-085	Northern Ireland
	CNET-086	United Kingdom
	CNET-091; CNET-092	Denmark
Ovine (<i>n</i> = 3)	CNET-104; CNET-106; CNET-107	United Kingdom
Canine (<i>n</i> = 3)	CNET-111; CNET-112; CNET-113	Sweden

CNET: CAMPYNET collection.

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