COMUNICAZIONE 5

PRELIMINARY STUDY ON CYTOLETHAL DISTENDING TOXIN (CDT) ACTIVITY IN *CAMPYLOBACTER JEJUNI* ISOLATED IN ITALY

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Risultati preliminari sull'attività della cytolethal distending toxin (CDT) in ceppi di *Campylobacter jejuni* isolati in Italia

Parole chiave: *Campylobacter jejuni*, pollame, carni avicole, uomo, CDT

Riassunto: Nonostante i dati epidemiologici internazionali confermino l'importanza di *Campylobacter jejuni* quale patogeno tossinfettivo umano, le conoscenze riguardo ai meccanismi di patogenicità rimangono ancora controverse e limitatei. I principali fattori di virulenza a tutt'oggi conosciuti sono la motilità, l'aderenza, la capacità di invasione e la produzione di tossine. Lo scopo di questo lavoro è stato quello di studiare la produzione della tossina CDT (cytolethal distending toxin) in un gruppo di 29 isolati di *Campylobacter jejuni* isolati da diverse fonti (uomo, animali, alimenti ed ambiente) precedentemente caratterizzati mediante ribotipizzazione automatica. I risultati ottenuti hanno evidenziato un diverso livello di produzione di CDT in tutti gli isolati ad eccezione del ceppo n° 3 negativo per il test adottato. Il sequenziamento dei geni *cdt* in tale isolato ha evidenziato 16 sostituzioni nucleotiche tre delle quali responsabili delle sostituzioni aminoacidiche che potrebbero essere alla base della mancata attività citotossica. Nessuna correlazione è stata osservata tra ribotipo, fonte di isolamento e livello di tossina prodotta.

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Introduction

Cytolethal distending toxin (CDT) production by *Campylobacter jejuni* was first described by Johnson and Lior in 1988 (3). CDT activity in culture supernatants caused several cultured cell lines, including HeLa and Vero cells, to become slowly distended over a 2- to 4- day period, after which the cell disintegrated and died (3). The *C. jejuni cdt* genes have been cloned and sequenced (4). CDT activity is encoded by three adjacent or slightly overlapping genes, *cdt*A, *cdt*B, *cdt*C, which encode proteins predicted to have molecular weights of about 30,000, 29,000, and 21,000, respectively. The specific function of the CDT proteins are unknown, the predicted amino acid sequences are unlike those of any of the proteins in the available database (4). Lara-Tejero et al. (1) recently reported that the three sub-units, when combined, interact with one other to form an active tripartite holotoxin. The actual target of the CDT remains undiscovered, however *C. jejuni* CDT causes HeLa cells to become blocked in the G_2 phase of the cell cycle (5).

The aim of this study was to investigate the cytolethal distending toxin (CDT) activity of 29 *Campylobacter jejuni* isolated from different sources and previously characterised by automatic *Pst*I riboprinting.

Materials and Methods

Twenty-nine *Campylobacter jejuni* isolates from human, animal and food sources (Table 1), were screened with an *in vitro* HeLa cell cytolethal distending toxin assay (2,4): all of them were previously characterised with automatic *Pst*I riboprinting.

Table 1- Campylobacter isolates in relation to different sources
Tabella 1- Isolati di Campylobacter in relazione all'origine

Nr.	SOURCE	RG	Nr.	SOURCE	RG
1	Broiler	103 S1	16	Poultry product	32 S1
2	Human	103 S1	18	Human	36 S5
3	Broiler	103 S3	19	Poultry product	36 S5
4	Human	103 S3	20	Poultry product	44 S5
5	Human	104 S1	21	Poultry product	44 S5
6	Poultry product	104 S1	22	Human	45 S2
7	Turkey	23 S2	23	Human	48 S4
8	Human	23 S2	24	Human	66 S2
9	Turkey	23 S4	25	Human	66 S3
10	Human	23 S4	26	Poultry slaughterhouse	67 S2
11	Human	26 S2	27	Poultry slaughterhouse	67 S5
12	Human	26 S2	28	Poultry product	99 S4
13	Poultry slaughterhouse	26 S2	29	Poultry product	99 S4
14	Human	30 S3	30	Poultry product	99 S4
15	Human	30 S3			

All the samples were tested with a PCR to screen for the *cdt* genes. All the isolates gave a PCR product of the expected size of 2.14 kb.

For the toxin assay, Campylobacter strains were grown on Muller Hinton plates for 24 hrs at 42°C in microaerobic atmosphere (10% CO_2, 85% N_2, 5% O_2) and harvested into Eagles Minimal Essential Medium.

The cell suspensions were measured at the OD_{550}, in triplicate and adjusted until the CDT titre had a range of OD_{550} and applied, in a range of dilutions, to HeLa cells ($2x10^4$ cells/ml) and incubated for 5 days at 37°C in 5% of CO_2. The treated monolayers were fixed, stained with crystal violet and examined with the microscope at x10 magnification. The toxin titre was defined as the reciprocal of the highest dilution of a preparation that affected 50% of the HeLa cells in a well of a 96- well assay plate. In the figure 1, the effect of CDT on HeLa cells is shown. Each strain was assayed in at least three independent assays and the reference strain *C. jejuni* NCTC 1168 was included.

Figure 1 - Effect of CDT on HeLa cells
Figura 1 - Effetto della CDT sulle cellule HeLa

HeLa cells before
osure to C. jejuni CDT

Results and Discussion

As expected, the majority of *Campylobacter jejuni* strains make CDT, although the amounts made by different isolates can vary, as show in the figure 2. Moreover a significant variation in the toxin activity was observed among the 29 strain tested compared with the source and the ribogroup. Any correlation was observed between the ribotype, the source of isolates and the toxin activity. In fact, as show in previous research (4), the high toxin productions can't be associated with *Campylobacter jejuni* that cause diarrheal disease in human.

For the sample nr.3, negative for the toxin assay, reverse transcriptase PCR was used to confirm that the *cdt* genes of the strain are expressed. DNA sequencing was used to detect the difference between the nucleic sequence of the strain nr.3 compared with the reference strain *C. jejuni* NCTC 1168. 16 nucleotide substitutions were found within the sample nr.3 when compared with the sequence of the strain 1168. Three of these resulted in an amino acid change: at position 88 in *cdt*A Ala>Val, at position 120 in *cdt*B Met>Thr and at the position 167 in *cdt*C Ile>Asn.

Expression analysis indicated that the genes are co-transcribed and therefore one or more of these changes in the *cdt* genes may be associated with loss of toxin activity in this strains.

HeLa cells after 5 days
exposure to C. jejuni CDT

References

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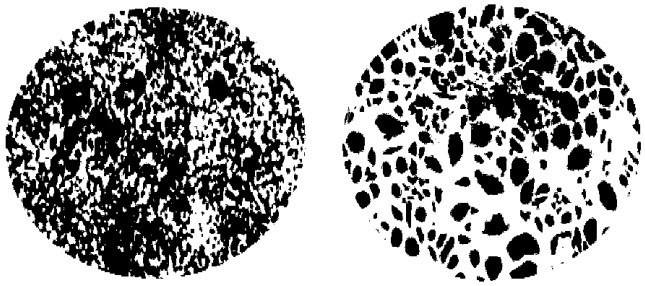

Figure 2 - Comparison of the CDT activities
Figura 2 - Comparazione dell'attività della CDT