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ScienceDirect
Comparative Immunology, Microbiology
and Infectious Diseases 33 (2010) 355–364

COMPARATIVE
IMMUNOLOGY
MICROBIOLOGY &
INFECTIOUS
DISEASES

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Prevalence of virulence-associated genes and cytolethal distending toxin production in *Campylobacter* spp. isolated in Italy

Giancarlo Ripabelli[a,*], Manuela Tamburro[a], Fabio Minelli[b],
Annalisa Leone[a], Michela Lucia Sammarco[a]

[a] *Department of Health Sciences, Faculty of Medicine and Surgery, University of Molise,*
Via De Sanctis, 86100 Campobasso, Italy
[b] *Department of Veterinary Public Health and Food Safety, Istituto Superiore di Sanità,*
Viale Regina Elena, Rome, Italy

Accepted 22 December 2008

Abstract

The prevalence of virulence and cytolethal distending toxin (CDT) genes and the cytotoxic activity in Vero and HEp-2 cells was estimated in 29 *Campylobacter jejuni* and 36 *Campylobacter coli* from foods, animals and humans isolates. All *C. jejuni* showed *fla*A, *cad*F, *cdt*A, *cdt*B, *cdt*C and *cdt* cluster genes fragments, except for *ceu*E (86.2%) and *cdt* genes (93.1%). Amongst *C. coli* strains, a lower prevalence of *ceu*E gene (83.3%) was detected than that for *cdt*A, *cdt*B, *cdt*C genes (97.2%), *cdt* gene cluster (94.4%) and *cdt* genes (86.1%); whereas *fla*A and *cad*F genes were amplified in all isolates. Despite the high prevalence of CDT genes only 8 (27.6%) *C. jejuni* and 1 (2.8%) *C. coli* showed evidence for cytotoxin production in HEp-2 cells. However, how CDT positive and CDT negative strains differ in their biological properties remains unknown, but the relative higher prevalence of citotoxicity in *C. jejuni* could be consistent with its predominant epidemiological role in human infections.

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* Corresponding author. Tel.: +39 0874 404961; fax: +39 0874 404752.
E-mail address: ripab@unimol.it (G. Ripabelli).

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doi:10.1016/j.cimid.2008.12.001

Résumé

La prévalence des gènes codant pour les toxines et les facteurs de virulence ainsi que l'activité cytotoxique dans les cellules Vero et HEp-2 ont été évaluées avec 29 *Campylobacter jejuni* et 36 *Campylobacter coli* provenant d'aliments, d'animaux et de cas humains. Tous les isolats de *C. jejuni* ont montré la présence des gènes *fla*A, *cad*F, *cdt*A, *cdt*B, *cdt*C et *cdt* cluster, sauf pour *ceu*E (86,2%) et *cdt* gènes (93,1%). Parmi les isolats de *C. coli*, une plus faible prévalence du gène *ceu*E (83,3%) a été observée par rapport aux gènes *cdt*A, *cdt*B et *cdt*C (97,2%), *cdt* gène cluster (94,4%) et *cdt* gènes (86,1%); tandis que les gènes *fla*A et *cad*F ont été amplifiés dans tous les isolats. Malgré la prévalence élevée des gènes codant la toxine CDT, seulement 8 (27,6%) *C. jejuni* et 1 (2,8%) *C. coli* ont mis en evidence la production de toxines dans les cellules HEp-2. Toutefois, la différence des propriétés biologiques entre CDT positifs et négatifs reste méconnues, mais la plus forte prévalence de toxicité en *C. jejuni* peut etre corrélée avec son rôle épidémiologique prépondérant dans les infections humaines.

Mots clés : *Campylobacter* spp. ; épidémiologie ; toxine et virulence genes ; Cytolethal distending toxine*Keywords:* *Campylobacter* spp.; Epidemiology; Virulence and toxin genes; Cytolethal distending toxin

1. Introduction

Campylobacteriosis is one of the most common bacterial causes of gastroenteritis worldwide [1]. However, specific virulence mechanisms are not fully elucidated, although flagella mediated-motility, adhesion to intestinal mucosa, invasion and production of enterotoxin and cytotoxin have been identified as virulence determinants [2,3]. A number of putative virulence and toxin genes have been studied, including: *fla*A and *cadF* genes involved in adhesion and colonization of the host's intestine [4,5]; *ceu*E gene which encodes a binding-protein transport system for the siderophore enterochelin [6,7]; *cdt* gene cluster consisting of three adjacent genes (*cdt*A, B and C). The cytolethal distending toxin (CDT) is composed of CdtB as the enzymatically active subunit, and the two heterodimeric subunits CdtA and CdtC responsible for the holotoxin binding to cell membrane [8]. *Campylobacter* spp. cytotoxin causes DNA lesions, cromatine fragmentation, cytoplasm distension and cell cycle arrest in the G_2/M transition phase, leading to progressive cellular distension and, ultimately, death in several cell lines [9–11].

The aim of this study was to estimate the prevalence of virulence (*cad*F, *fla*A, *ceu*E) and CDT toxin encoding genes (*cdt*A, *cdt*B, *cdt*C) in *Campylobacter jejuni* and *Campylobacter coli* isolated in Italy from different sources, and to correlate the presence of these genes with cytotoxic activity using *in vitro* tests on Vero (African green monkey kidney epithelial) and HEp-2 (epithelial human carcinoma) cell lines. Nowadays, in a globalisation era, the understanding of infectious disease epidemiology can be completely achieved by a global view of the epidemiological situation. Hence, the evaluation of the pathogenic potential of *C. jejuni* and *C. coli* isolates could further assist in the assessment of particular animal reservoirs and/or strains as likely sources for human infection.