Eur J Clin Microbiol Infect Dis (2001) 20:676–677
DOI 10.1007/s100960100571

BRIEF REPORT

C. Abrahao · R.J. Carman · H. Hahn · O. Liesenfeld

Similar Frequency of Detection of *Clostridium perfringens* Enterotoxin and *Clostridium difficile* Toxins in Patients with Antibiotic-Associated Diarrhea

Published online: 14 September 2001

Antibiotic-associated diarrhea (AAD) continues to be a major nosocomial health problem [1, 2]. *Clostridium difficile* toxins can be detected in 10–25% of patients with AAD, but no etiologic agent can be identified in up to 75% of cases [1, 2]. In the early 1980s, *Clostridium perfringens* enterotoxin (CPEnt) was identified as a cause of some cases of AAD [3, 4]. However, the literature lacks detailed prospective studies on the prevalence of CPEnt in patients with AAD [5, 6]. In the present report, we investigated the prevalence of CPEnt in stool samples submitted for detection of *Clostridium difficile* toxins.

A total of 242 consecutive stool samples collected from 156 patients and submitted to the Department of Medical Microbiology and Immunology of Infection for detection of *Clostridium difficile* toxins between March and June 1998 were analyzed. CPEnt was detected using an enzyme-linked immunoassay (ELISA) (*C. perfringens* Enterotoxin Test; TechLab, USA). This ELISA uses two polyclonal antibodies specific for CPEnt. Although there is no gold standard for the detection of CPEnt, comparison of the CPEnt ELISA with a cytotoxicity assay on Vero cells revealed 100% correlation [6]. *Clostridium difficile* toxins A and B were detected using ELISA (*C. difficile* Tox A/B Test; TechLab). *Clostridium perfringens* and *Clostridium difficile* were grown on standard media and identified biochemically (Rapid ID32A; bioMérieux, Germany). In addition, the patient's history was evaluated by chart review. Control groups consisted of 50 stool samples collected from healthy volunteers and 75 stool samples submitted for the detection of enteropathogenic bacteria (salmonella, shigella) and/or ova/parasites (O/P). Statistical analysis was performed using Fisher's exact test; P values of <0.05 were considered significant.

CPEnt was detected in the stool samples of 10 (6.4%) patients. In contrast, CPEnt was not detected in stool samples from healthy volunteers or in samples submitted for the detection of enteropathogenic bacteria and/or O/P. *Clostridium difficile* toxins were detected in stool samples of 10 (6.4%) patients. Of these, one (0.6%) patient harbored both CPEnt and *Clostridium difficile* toxins.

All patients positive for CPEnt or *Clostridium difficile* toxins had either received antibiotic therapy or were severely immunosuppressed (endogenous or exogenous). Patients with CPEnt did not differ from patients with *Clostridium difficile* toxins in variables including sex, age, diarrhea, and frequency of prior antibiotic therapy (Table 1). However, severe immunosuppression was observed significantly more often in patients with CPEnt than in those with *Clostridium difficile* toxins. Primary diagnoses in patients with CPEnt included renal transplant (2 cases), HIV infection (stage B2 in 2 cases, stage A2 in 1), Crohn's disease, lymphoma, subarachnoid bleeding, and acute myeloic leukemia (1 case each). Patients with *Clostridium difficile* toxins presented with urinary tract infection, diabetes and chronic obstructive lung disease, cirrhosis of the liver, pneumonia, stomach ulcers, sepsis under chemotherapy-induced agranulocytosis, sepsis following renal transplantation, and hip surgery (1 each). One patient who harbored both CPEnt and *Clostridium difficile* toxins presented with peptic ulcer disease following renal transplantation; this patient had developed diarrhea following antibiotic and immunosuppressive therapy.

Patient variables also did not differ between patients with CPEnt and/or *Clostridium difficile* toxins and those without CPEnt and/or *Clostridium difficile* toxins (data not shown).

We observed an inverse relationship between the frequency of detection of CPEnt and the frequency of isolation of *Clostridium perfringens*. Culture for *Clostridium perfringens* yielded positive results in 4.1% of stool sam-

C. Abrahao · H. Hahn · O. Liesenfeld (✉)
Department of Medical Microbiology
and Immunology of Infection, Benjamin Franklin Medical Center,
Free University of Berlin, 12203 Berlin, Germany
e-mail: olitoxo@zedat.fu-berlin.de
Tel.: +49-30-84453630, Fax: +49-30-84453830

R.J. Carman
TechLab Inc., Blacksburg, VA 24060-6364, USA

Table 1 Comparison of personal and clinical characteristics of patients positive for CPEnt or *Clostridium difficile* toxins in stool samples

Patient characteristic	Positive result		*P* value
	CPEnt (n =9)[a]	*Clostridium difficile* toxins (n =9)[a]	
Female/male	3/6	5/4	N.S.
Mean age in years (range)	54.2 (39–68)	65.2 (46–89)	N.S.
Diarrhea (%)	7/9 (77.7)	8/8[b] (100.0)	N.S.
Antibiotic therapy (%)	7/9 (77.7)	7/8[b] (87.5)	N.S.
Severe immunosuppression (%)	8/9 (88.9)	2/8[b] (25.0)	0.0152

N.S., not significant
[a] One patient (CPEnt- and *Clostridium difficile* toxins-positive) was excluded from the analysis
[b] Information not available for one patient

ples submitted for detection of *Clostridium difficile* toxins, 18% in stool samples submitted for detection of enteropathogenic bacteria and O/P, and 40% in those obtained from healthy volunteers. In contrast, CPEnt was detected in 4.1% of samples submitted for detection of *Clostridium difficile* toxins and was not detected in stool samples submitted for detection of enteropathogenic bacteria and O/P and stool samples obtained from healthy volunteers. These results most likely reflect the lower rate of antibiotic usage and carriage of nontoxigenic strains of *Clostridium perfringens* (whose natural habitat is the gastrointestinal tract) in the latter group.

In conclusion, the present study reveals detailed information about the prevalence of CPEnt in Europe and characteristics of patients with CPEnt-associated AAD. These results thus confirm earlier research indicating a role for CPEnt in the etiology of AAD. In fact, in our institution CPEnt appears to be as important as *Clostridium difficile* toxins. Patients with CPEnt do not appear to differ in their personal and clinical characteristics from those with *Clostridium difficile* toxins. Since the rate of detection of CPEnt was shown to be as high as the rate of detection of *Clostridium difficile* toxins, we believe that detection of CPEnt should be included in the diagnostic work-up of patients with AAD. In those institutions that run both ELISAs for detection of *Clostridium difficile* toxins and CPEnt, all samples could be screened with one assay; thereafter, all negative samples could be run in the second assay. This technique would encompass the risks that a sample having both sets of toxins would be tested in only one assay. Boriello and Williams [7] reported successful treatment of AAD caused by CPEnt with metronidazole. Therefore, since treatment recommendations will be the same for patients with AAD caused by *Clostridium difficile* or *Clostridium perfringens* or *Clostridium difficile* and *Clostridium perfringens*, the screening approach may prove effective.

References

1. Bartlett J: *Clostridium difficile*: history of its role as an enteric pathogen and the current state of knowledge about the organism. Clinical Infectious Diseases (1994) 18:265–272
2. Kelly CP, Pothoulakis C, LaMont JT: *Clostridium difficile* colitis. New England Journal of Medicine (1994) 330:257–262
3. Borriello SP, Larson HE, Welch AR, Barclay F, Stringer MF, Bartholomew BA: Enterotoxigenic *Clostridium perfringens*: a possible cause of antibiotic-associated diarrhoea. Lancet (1984) 1:305–307
4. Borriello SP, Welch AR, Larson HE, Barclay F: Diarrhoea and simultaneous excretion of *Clostridium difficile* cytotoxin and *C. perfringens* enterotoxin. Lancet (letter) (1984) 2:1218
5. Boone JH, Carman RJ: *Clostridium perfringens*: food poisoning and antibiotic-associated diarrhea. Clinical Microbiology Newsletter (1997) 19: 965–969
6. Carman RJ, Boone JH, Woodburn MA, Evans DT, Hahn PE, Alexander LA: ELISA test for *Clostridium perfringens* enterotoxin (CpE) in cases of antibiotic associated diarrhea. Programm and Abstracts of the 99th Annual Meeting of the American Society for Microbiology, Washington, DC. American Society for Microbiology (1999)
7. Boriello SP, Williams RKT: Treatment of *Clostridium perfringens* enterotoxin-associated diarrhoea with metronidazole. Journal of Infection (1985) 10:65–67