

# CLOSTRIDIUM DIFFICILE INFECTIONS IN HIV-POSITIVE PATIENTS

Siripan Wongwanich, Sunanta Ramsiri, Mayura Kusum and Paijit Warachit

National Institute of Health, Nonthaburi, Thailand

**Abstract.** The prevalence of *Clostridium difficile* infections in HIV-positive patients with regard to the presence of its enterotoxin was investigated. Enzyme immunoassay (EIA, Meridian Diagnostic Inc) was used for the detection of *C. difficile* enterotoxin in stool specimens collected from 201 HIV-positive and 271 HIV-negative diarrheal patients. Culture was performed on cycloserine cefoxitin fructose agar. Chromosomal DNA types of *C. difficile* isolates were determined by pulsed-field gel electrophoresis (PFGE). In the HIV-positive group, *C. difficile* enterotoxin was found in 58.8% and 12.6% of diarrheal and non-diarrheal patients, respectively, whereas this toxin was found in 36.5 % of HIV-negative-diarrheal patients. However, 13.6% of stool samples were negative by toxin assay, but were positive for *C. difficile* by culture and latex agglutination test. Among 11 isolates from both HIV-positive and HIV-negative patients, 6 patterns of PFGE type were observed: A, B, C, D, E and F.

## INTRODUCTION

In recent year, several investigators showed that 11-12.5% of *C. difficile* associated diarrhea were found in HIV-positive patients (Barbut *et al*, 1993; Hutin *et al*, 1993; Mastroianni *et al*, 1997). Most of the patients had recently received antibiotics or antiviral agents before developing *C. difficile* diarrhea. The role of *C. difficile* in HIV-positive patients has not been defined in Thailand. The purpose of our study, therefore, was to investigate the prevalence of *C. difficile* in both HIV-positive and non-HIV infected patients with regard to the presence of its enterotoxin.

## MATERIALS AND METHODS

### Patients and stool specimens

A total of 472 fresh stool specimens were collected from 201 HIV-positive patients and from 271 non-HIV-infected patients. These included both HIV and non-HIV patients who developed diarrhea either during or after 2 weeks antibiotic administration, patients with non-antibiotic associated diarrhea and HIV-positive patients who had no diarrheal symptoms.

### Enterotoxin assay

The specific *C. difficile* enterotoxin in stool

This work was presented in part at the 9<sup>th</sup> European Congress of Clinical Microbiology and Infectious Diseases, Berlin, March 1999.

specimens were examined by using Premier Enzyme Immunoassay kit (Meridian Diagnostic Inc, Ohio, USA).

### Culture

All stool specimens were cultured for *C. difficile* by the use of a selective medium, cycloserine cefoxitin fructose agar (CCFA, Oxoid) and alcohol shock as previously described (Wongwanich *et al*, 1990).

### Detection of *C. difficile* antigen

The presence of *C. difficile* antigen in stool specimens were examined by using CD-D1 latex kit (Mitsubishi Chemical Industries, Tokyo).

### PFGE assay

DNA extraction and PFGE of 11 *C. difficile* isolates were performed by a modification of the method described by Smith *et al* (1988). Briefly, *C. difficile* cells grown overnight in 20 ml of brain heart infusion broth were resuspend in TES buffer to achieve a McFarland turbidity of 3 and mixed with an equal volume of 1.6 % low melting agarose (Bio-Rad Laboratories, Richmond, CA, USA) to make plugs for PFGE. The cells in the plugs were treated with lysis solution and then with protease. The DNA was digested overnight with *Sma*I (New England Biolabs Inc, Beverly, MA, USA). For PFGE, a CHEF DR II system (Bio-Rad Laboratories, Richmond, CA, USA) was used with switch time 5 and 30 seconds for 24 hours at 6 V/cm. The gels were stained with ethidium bromide and

photographed under UV light. Chromosomal DNA of *Saccharomyces cerevisiae* (Bio-Rad Laboratories) was used for molecular standard markers.

**Statistical analysis**

Data were analysed using Epi Info Software version 6. Categorical variables were compared using chi-square test. A p-value of < 0.05 was considered significant, and all tests were two-tailed.

**RESULTS**

In the HIV-positive patient group, *C.difficile* enterotoxin was detected in 58.8% (20/34) of diarrheal patients and 12.6% (21/167) in non-diarrheal patients, whereas it was found in 36.5% (99/271) of non-HIV infected diarrheal patients. There was a significant difference in the detection of enterotoxin in stool specimens collected from HIV-positive diarrheal patients and non-HIV infected diarrheal patients (p = 0.012).

The results of the *C. difficile* enterotoxin assay, latex agglutination and culture from stool specimens are summarized in Table 1. We found that in stool samples of 25.7% of patients contained enterotoxin without *C. difficile* antigen (LA-, EIA+). *C. difficile* could not be isolated from 15.2% of stool specimens which contained the *C. difficile* antigen. However, there were no specimens that gave a negative antigen but were positive for organism.

The DNA patterns obtained with PFGE analysis are shown in Figs 1 and 2. Among 11 *C. difficile*

isolates, 6 patterns of DNA type (A, B, C, D, E and F) were observed. Four isolates were D type, 3 isolates were A type. The others four isolates were B, C, E and F type. Two Isolates (no. 326 and no. 348) were recovered from the same non-HIV infected patient. The second was isolated 18 days after the first.

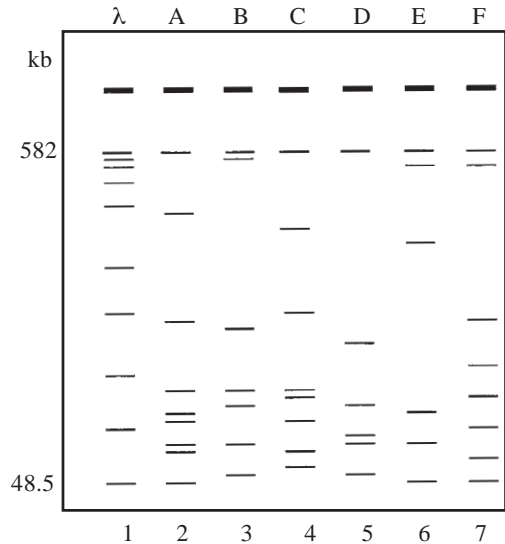


Fig 1—A schematic presentation of PFGE patterns of *Sma*I-digested chromosomal DNA of *C. difficile*. The PFGE types are shown above the lanes.

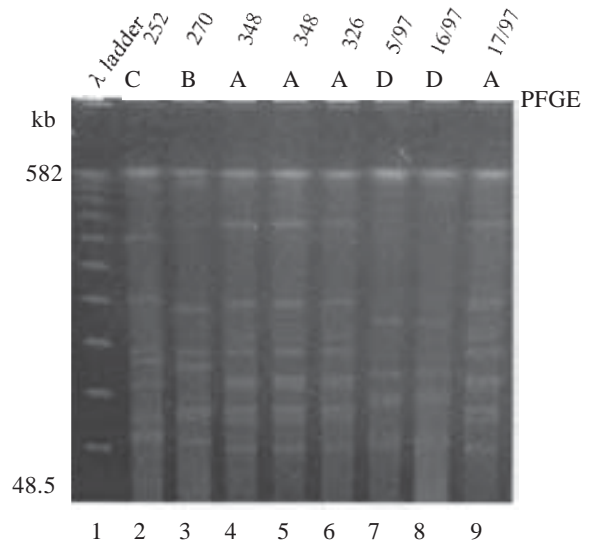


Fig 2—DNA patterns obtained by PFGE of *C. difficile* genomic DNA digested with *Sma*I. The number at the top of the panel refer to the number of strains.

Table 1

Results of the enterotoxin assay (EIA), CD-D1 latex agglutination test (LA) and culture (CD) from stool specimens collected from both HIV and non-HIV infected patients.

No. of specimens	Result of test	%
214	LA+, EIA+	12.6
	LA-, EIA+	25.7
	LA+, EIA-	13.6
270	LA+, CD+	7.0
	LA+, CD-	15.2
	LA-, CD+	0
214	LA+, EIA+, CD+	7.0

PFGE assay demonstrated a good discriminative capacity for investigating the relatedness of isolates recovered from different patients (isolate no. 5/97 and no. 16/97 showed the same PFGE type D) and the identities of isolates from the same patient (isolate no. 326 and no. 348 showed the same PFGE type A).

## DISCUSSION

In the present study we demonstrated that the prevalence of *C. difficile*-associated diarrhea in HIV-positive patients were higher in HIV-positive (58.8%) than non-HIV infected (36.5%) diarrheal patients ( $p < 0.05$ ). Although HIV infection itself is not considered an intrinsic risk factor for *C. difficile* infection, individuals infected with HIV appear to be at increased risk for *C. difficile*-associated diarrhea. Because patients with HIV spend considerable time in hospitals and are given antimicrobial therapy.

Because of the increasing incidence of nosocomial diarrhea caused by *C. difficile*, there is a need for an effective procedure for typing these bacteria in order to obtain information about sources and routes of transmission. PFGE has been successfully applied to differentiate *C. difficile* strains. In this study, PFGE assay demonstrated a good discriminative capacity for investigating the relatedness of isolates recovered from different patients (isolates no. 5/97 and no. 16/97 showed the same PFGE type D) and the identities of isolates from

the same patient (isolates no. 326 and no. 348 showed the same PFGE type A).

Our study demonstrated that in HIV-positive patients, *C. difficile* infection rate was ~2-times higher than that of non-HIV-infected patients. Clinicians should keep this pathogenic bacteria in mind when searching for the cause of diarrhea in HIV-positive patients.

## REFERENCES

- Barbut F, Depitre C, Delmee M, Corthier G, Petit JC. Comparison of enterotoxin production, cytotoxin production, serogrouping, and antimicrobial susceptibility to *Clostridium difficile* strains isolated from AIDS and human immunodeficiency virus-negative patients. *J Clin Microbiol* 1993; 31: 740-2.
- Hutin Y, Molina JM, Casin I, *et al.* Risk factors for *Clostridium difficile* -associated diarrhea in HIV-infected patients. *AIDS* 1993; 7: 1441-7.
- Mastroianni A, Coronado O, Nanetti A, Valentini R, Manfredi R, Chiodo F. Nosocomial *Clostridium difficile* -associated diarrhea in patients with AIDS : A three-year survey and review. *Clin Infect Dis* 1997; 25(suppl) : S204-5.
- Smith, CL, Klco SR, Cantor CR. Pulsed-field gel electrophoresis and the technology of large DNA molecules, In: Davies KE, ed. Genome analysis: a practical approach. Washington DC: IRL Press, 1988: 41-72.
- Wongwanich S, Ramsiri S, Vanasin B, Khowsaphit P, Tantipatayangkul P, Phan-urai R. *Clostridium difficile*-associated diseases in Thailand. *Southeast Asian J Trop Med Public Health* 1990; 20: 367-72.