Diarrhea recurrence in patients with *Clostridium difficile*-associated diarrhea: Role of concurrent antibiotics

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OBJECTIVE: To monitor prospectively patients with *Clostridium difficile*-associated diarrhea (CAD) in a six hundred bed tertiary care hospital to determine which factors influenced the recurrence of the diarrhea.

DESIGN: A prospective, nonrandomized study. After an initial diagnosis of CAD, patients were interviewed, and each week stool samples and environmental samples were monitored for the presence of toxigenic *C difficile* for as long as the patients remained in hospital. The relationship of concurrent antibiotics, prolonged fecal excretion of organism or toxin, and environmental contamination was assessed.

PATIENTS: Over a two-and-a-half year period, 75 consecutive patients with CAD were selected and those who gave their written informed consent were enrolled. A control group to evaluate environmental contamination consisted of 75 patients with diarrhea not associated with *C difficile*.

RESULTS: Of the 75 CAD patients, 11 (14.7%) had a recurrence of their diarrhea. Diarrhea recurrence was associated with an increased rate of prolonged excretion of toxigenic organism and/or *C difficile* toxin(s) (nine of 11 [81.8%] compared with nine of 64 [14.1%]; $P = 0.0001$; relative risk 14.25; 95% CI 3.383 to 60.023). The risk of diarrhea recurrence was not related to a specific antibiotic but to concurrent therapy. Treatment within 30 days of initial CAD-specific treatment with an antibiotic other than metronidazole or vancomycin occurred significantly more frequently in patients with recurrence of diarrhea compared with those who did not have a recurrence (eight of 11 [72.7%] compared with 22 of 64 [34.4%], $P = 0.022$; relative risk 4; 95% CI 1.153 to 13.881). The environmental contamination rate for toxigenic *C difficile* in week one in the rooms of patients with diarrhea not caused by *C difficile* was low (two of 75 [2.6%]) compared with week one data for patients with CAD (14 of 75 [18.7%], $P = 0.002$; relative risk 1.922; 95% CI 1.479 to 2.498). The most frequent site contaminated was the bedpan sprayer (eight of 14 [57.1%]). Pulsed field gel electrophoresis analysis of stool and environmental toxigenic isolates indicated that there was not a single endemic strain of *C difficile*.

CONCLUSIONS: This study indicates that the recurrence of diarrhea may be related to concurrent 'other' antibiotics. Although data indicated that there was a correlation between diarrhea recurrence and prolonged fecal excretion of toxin, further studies are required to clarify the clinical significance.

Key Words: *Clostridium difficile*; Diarrhea; Diarrhea recurrence; Nosocomial infection

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Récidive de la diarrhée chez les patients atteints de diarrhée associée à *Clostridium difficile* : rôle des antibiotiques concomitants

OBJECTIF : Surveiller de façon prospective les patients atteints de diarrhée associée à *Clostridium difficile* (DAC) dans un hôpital de soins tertiaires de six cents lits pour déterminer quels facteurs ont influencé la récurrence de la diarrhée.

MODÈLE : Étude prospective, non randomisée. Après un diagnostic initial de DAC, les patients ont été interviewés et, chaque semaine, pendant toute la durée du séjour hospitalier des patients, on a recherché la présence de *C. difficile* toxigène dans les échantillons de selles et dans les échantillons environnementaux. On a aussi évalué la relation entre les antibiotiques concomitants, l'excrétion fécale prolongée de l'organisme ou de la toxine et la contamination environnementale.

PATIENTS : Sur une période de deux ans et demi, 75 patients consécutifs atteints de DAC ont été sélectionnés et ceux qui ont donné leur consentement éclairé par écrit ont été admis dans l'étude. Un groupe témoin servant à évaluer la contamination environnementale consistait en 75 patients atteints de diarrhée non associée à *C. difficile*.

RÉSULTATS : Des 75 patients atteints de DAC, 11 (14,7 %) ont subi une récurrence de leur diarrhée. Cette dernière était associée à une augmentation du taux d'une excrétion prolongée de l'organisme toxigène et/ou des toxines de *C. difficile* (neuf sur 11 [81,6 %] comparativement à neuf sur 64 [14,1 %] ; $p = 0,0001$; risque relatif de 14,25 ; IC à 95 % : 3,383-60,023). Le risque de récurrence de la diarrhée n'était pas relié à un antibiotique spécifique, mais à un traitement concomitant. L'administration d'un traitement dans les 30 jours après l'amorce d'un traitement spécifique pour la DAC avec un antibiotique autre que le métronidazole ou la vancomycine était beaucoup plus fréquente chez les patients souffrant d'une récurrence de leur diarrhée comparativement à ceux ne souffrant pas d'une récurrence (huit sur 11 [72 %] comparativement à 22 sur 64 [34,4 %], $p = 0,022$; risque relatif de 4 ; IC à 95 % : 1,153-13,881). Le taux de contamination environnementale pour *C. difficile* toxigène à la semaine 1 dans les chambres des patients atteints de diarrhée non causée par *C. difficile* était faible (deux sur 75 [2,6 %] comparativement aux données de la semaine 1 chez les patients atteints de DAC (14 sur 75 [18,7 %], $p = 0,002$; risque relatif de 1,922 ; IC à 95 % : 1,479-2,498). Le site le plus souvent contaminé était la douche du bassin hygiénique (huit sur 14 [57,1 %]). Une analyse par électrophorèse sur gel en champ pulsé des selles et des isolats toxigènes provenant de l'environnement a démontré qu'il n'y avait pas une seule souche endémique de *C. difficile*.

CONCLUSIONS : La présente étude indique que la récurrence de la diarrhée pourrait être reliée aux « autres » antibiotiques concomitants. Bien que ces données aient démontré une corrélation entre la récurrence de la diarrhée et l'excrétion fécale prolongée de la toxine, des études plus approfondies sont nécessaires pour en clarifier la portée clinique.

Clostridium difficile is the most commonly known etiological agent of hospital-acquired infectious diarrhea (1-7). Only strains that produce both toxin A and toxin B have been associated with human disease (7-16). Approximately 15% (range of 5% to 24%) of patients who are treated for *C. difficile*-associated diarrhea have a recurrence of their diarrhea (17-19); this may be due to relapse with their original *C. difficile* strain or due to reinfection with a different strain (20). The basis for the recurrence of diarrhea and the role of prolonged fecal excretion of toxin in the risk of diarrhea recurrence have not been well studied. The aims of this study were to follow prospectively 75 patients with *C. difficile*-associated diarrhea (CAD), and determine the frequency and temporal distribution of diarrhea recurrence; determine whether factors such as concurrent antibiotics, or continued excretion of *C. difficile* toxin(s) or toxigenic organisms in patients treated for CAD correlated with diarrhea recurrence; and determine the frequency of environmental contamination and the relationship of environmental isolates to patient isolates.

PATIENTS AND METHODS

Patient populations studied: Inpatients of St Boniface General Hospital, St Boniface, Manitoba, with diarrhea, whose stool samples had been tested for *C. difficile* cytotoxin, were reviewed on a daily basis. The routine CAD diagnostic test was the tissue culture cytotoxin assay. Patients were arbitrarily selected on a consecutive basis and asked to give written informed consent for enrolment in the study. Human experimentation guidelines of the United States Department of Health and Human Services, and the University of Manitoba

were followed in the conduct of this research. These patients were not matched for any criteria other than the presence of clinically significant diarrhea. These patients were prospectively monitored during their hospital admission (to a maximum of 13 weeks). Relevant clinical data were obtained from chart reviews and weekly interviews with the patients. Weekly swab cultures were obtained from stools, bedrail, buzzer, toilet (or commode as appropriate) and bedpan sprayer. Weekly stool samples obtained after enrolment were evaluated by ELISA testing to detect toxins A and B, by tissue culture for cytotoxin and by culture for viable organisms. One toxigenic isolate or sample was stored and used for pulsed field gel electrophoresis (PFGE) analysis.

To assess endemic environmental contamination with this organism, a group of control patients who were hospitalized and had diarrhea that was not associated with *C. difficile* were included in the study. The one-time environmental sampling included bedrail, buzzer, bedpan sprayer and toilet (or commode as appropriate) samples. None of the patients enrolled in this part of the study shared rooms with patients who had CAD.

Case definitions: The following case definitions were used throughout the study analysis.

CAD: CAD patients were symptomatic (ie, had diarrhea defined as stool that took the shape of the container and frequent enough for a physician to consider it clinically significant) with cytotoxin detected directly in the stool. In addition, patients had to have been treated with either vancomycin or metronidazole for their diarrhea or have had their previous antimicrobial(s) discontinued.

Diarrhea recurrence: For a patient to have a recurrence of diarrhea, the patient's diarrhea had to have stopped after treatment of the initial episode, recurred during the same hospitalization and met all of the criteria outlined in the definition of CAD. Patients whose diarrhea initially responded to metronidazole or vancomycin therapy, and subsequently recurred while still on this antimicrobial treatment, were included in this group.

Treatment failure: A treatment failure happened when the patient's diarrhea persisted and did not respond despite antimicrobial treatment with metronidazole or vancomycin.

Prolonged excreter: A prolonged excreter was a patient treated for CAD, but who continued to have detectable toxin(s) (by cytotoxin testing or ELISA) or grew a toxigenic *C difficile* isolate more than three weeks after initiation of specific therapy for CAD.

Bacterial strains and culture media: Stool and environmental samples were inoculated into thioglycollate broth containing 250 g/mL of cycloserine and 8 g/mL of cefoxitin (CCF Supplement, Oxoid, Nepean, Ontario). The inoculated cycloserine and cefoxitin broths (CCFB) were then incubated aerobically at 35°C. The CCFB acts as an enrichment broth for *C difficile* in stool samples. Any inoculated CCFB tubes that showed turbidity were subcultured onto prereduced cycloserine, cefoxitin, fructose agar (CCFA) plates and incubated in an anaerobic chamber for 48 h. The concentration of CCF supplement in the CCFA plates was the same as the CCFB. Colonies of *C difficile* were identified by Gram stain, fluorescence under ultraviolet light, colonial morphology and a positive latex-agglutination with *C difficile* specific antisera (Serobact, Wellmark Diagnostics, Guelph, Ontario). One isolate of *C difficile* from each site was tested for cytotoxin B production and then stored in skim milk at -70°C for subsequent PFGE analysis. Inoculated CCFB tubes showing no turbidity by five days were discarded as negative.

Cytotoxin B tissue culture assay: Stool samples were processed according to standard cytotoxin assay procedures using human foreskin fibroblast (HFF) cells. The final dilution of the stool sample in the HFF monolayer was 1:24. Appropriate positive and negative controls (Baxter Diagnostics, Mississauga, Ontario) for the *C difficile* assay were run with each tray. Monolayers were observed after 24 and 48 h incubation. Monolayers showing characteristic cytopathic effect (CPE) that was neutralizable by antitoxin were called positive for cytotoxin B. If no CPE was observed after 48 h incubation, the sample was reported as negative for cytotoxin B. Samples showing unusual looking CPE (ie, not characteristic rounding of cells) or CPE not neutralizable by antitoxin, were diluted further (final dilution of 1:50 or 1:100) and retested to rule out nonspecific CPE.

ELISA for toxin A and B: Stool samples were either processed immediately or stored at -70°C before the ELISA assay. The Cytoclon A+B EIA assay (Cambridge Biotech Corporation, Worcester, Massachusetts) was performed as described by the manufacturer.

PFGE – Chromosomal DNA isolation: Total genomic DNA was prepared using modifications of the method described by Simor et al (21). *C difficile* isolates were grown in 10 mL of

prereduced brain-heart infusion broth (BHI) with 20 mM L-threonine anaerobically at 35°C for 16 to 18 h. Cells were centrifuged and resuspended in 300 L PIV buffer (10 mM Tris-hydrochloric acid, pH 7.6 [Sigma Chemical Company, St Louis, Missouri], 1 M sodium chloride [Sigma]). An equal volume of 1.6% low-melt agarose (Gibco-BRL, Burlington, Ontario) was added and the suspension was mixed, pipetted into plug molds (Bio-Rad, Mississauga, Ontario) and allowed to set. Plugs were placed in 2 mL of lysis buffer (6 mM Tris-hydrochloric acid, pH 7.6, 1 M sodium chloride, 100 mM EDTA [Sigma], 0.5% Brij-58 [Sigma], 0.2% deoxycholate, 0.5% sarkosyl [Sigma], 20 g/mL RNase [Sigma], 2 mg/mL lysozyme [Sigma] and 2.5 g/mL mutanolysin [Sigma]) and incubated for 8 to 24 h at 37°C with gentle shaking. Plugs were then washed with TE buffer (10 mM Tris-hydrochloric acid, 0.1 mM EDTA pH 9.0) for 30 mins and then allowed to incubate at 50°C with gentle agitation in ESP buffer (0.5 M EDTA pH 9.0, 1% sarkosyl and 50 g/mL proteinase K [Boehringer-Mannheim, Laval, Quebec]). After digestion, plugs were washed three times with TE buffer and then stored at 4°C until use.

Restriction enzyme digestion and electrophoresis: Fragments of DNA generated after digestion using 20 U/plug of *Sma*I (Boehringer-Mannheim) were separated by PFGE using a CHEF Mapper system (Bio-Rad) in a 1% gel made with 0.5X Tris-borate-EDTA buffer (Sigma). The pulse times were ramped linearly from 1 s to 40 s over 22 h at 200 V and 12°C. Gels were stained with 0.5 g/mL of ethidium bromide and photographed using ultraviolet illumination.

Statistical analysis: All data were entered into a computer database, and statistical analysis was performed using the GraphPad InStat (GraphPad Software, San Diego, California) statistical program. The *t* test was used for continuous variables, and the χ^2 test or Fisher's Exact test were used for discrete variables. Nonparametric analysis (eg, for days in hospital) for unpaired data was done using the Mann-Whitney test.

RESULTS

One hundred and fifty patients gave informed consent; 75 patients with CAD were enrolled and monitored prospectively to determine whether diarrhea recurrence was related to concurrent antibiotics and/or prolonged excretion, and to assess environmental contamination. Seventy-five patients with diarrhea not due to *C difficile* were enrolled to determine the level of endemic environmental contamination only. Patients were enrolled over a 2.4-year period from November 1991 to January 1994. In 1991, before starting this study, the positivity rate for *C difficile* was 16.2 % (1249 submitted samples, of which 202 were positive), and the case incidence per 100,000 patient-days was 49.6. In each subsequent year, approximately one-third of all the patients with CAD were enrolled in the study. The case incidence in 1992 and 1993 was 48.6 and 35.2, respectively, per 100,000 patient days.

Stool samples from the 75 CAD patients were monitored prospectively (Figure 1). Up to 50% of CAD patients leave hospital within three to four weeks of being treated for CAD (Figure 1). Despite decreasing numbers of patients remaining in hospital after CAD therapy was initiated, each week about

Figure 1) Number of weeks that patients were followed in the study. Although 75 patients were enrolled in the study, over half were discharged by week 3. The distribution of when diarrhea recurrence occurred compared with the number of weeks that the patients were in the study is shown by the data at the bottom of the graph. Eleven patients had diarrhea recurrence

one-third of the patients continued to have stool samples that were test positive by one of the diagnostic tests evaluated. Eleven patients had diarrhea recurrence, and 18 of the 75 patients were prolonged excretors. The distribution of diarrhea recurrence stratified by week of hospitalization is shown in Figure 1. Comparative clinical information for diarrhea recurrence versus nondiarrhea recurrence CAD patients who were prospectively monitored is given in Table 1. The mean age and the ratio of men to women in the two populations were not significantly different; however, the range of ages was very wide for both groups (Table 1).

The majority of patients (93%) treated for CAD received metronidazole. The average length of initial metronidazole therapy was 10.9 ± 5.3 days and 7.5 ± 5.9 days for patients with diarrhea recurrence and nondiarrhea recurrence, respectively (not significantly different $P=0.08$). Thirteen patients were later switched to vancomycin for a number of reasons. To determine further whether the antibiotic therapy that these patients received affected on diarrhea recurrences, the time-line of diarrhea and antibiotic use was made (Figure 2). The average time to the first episode of diarrhea recurrence after completion of therapy for CAD (either metronidazole or vancomycin) was 11.5 days (range two to 39 days). It was not possible to determine whether the two cases of diarrhea recurrence that occurred while patients were taking their initial metronidazole therapy were due to treatment failure. The patients with diarrhea recurrence (eight of 11, 72.7%) were significantly more likely ($P=0.022$, relative risk 4.0, 95% CI 1.15 to

TABLE 1
Clinical information about patients with *Clostridium difficile*-associated diarrhea studied at St Boniface General Hospital

Clinical information	Patients with diarrhea recurrence (N=11)	Patients with no recurrence of diarrhea (N=64)	P
Women to men	7:4	40:24	1.000
Age (mean) (lower to upper 95% CI)	69.36 (56.8-81.9)	69.59 (66.3-72.8)	0.675*
Diabetes mellitus (%)	5 (45.5%)	17 (26.6%)	0.282
Laxative use (%)	5 (45.5%)	17 (26.6%)	0.282
Stool softeners (%)	4 (36.4%)	17 (26.6%)	0.489
Enema use (%)	4 (36.4%)	13 (20.3%)	0.257
Renal disease (%)	3 (27.3%)	19 (29.7%)	1.0000
Bowel movements/day in week 1; mean (lower to upper 95% CI)	5.09 (3.8-6.3)	4.87 (4.1-5.7)	0.414*
Mean days duration of first episode of diarrhea (lower to upper 95% CI)	3.54 (1.9-5.2)	8.05 (5.2-10.9)	0.108*
Blood in stool (visual) (%)	3 (27.3%)	10 (15.6%)	0.391
Days in hospital before enrolment (mean) (lower to upper 95% CI)	19.36 (10.2-28.6)	35.48 (21.7-49.3)	0.385*
Days in hospital after enrolment (mean) (lower to upper 95% CI)	65.45 (20.0-110.9)	32.68 (15.6-49.8)	0.034*
Number (%) in room where <i>C difficile</i> grown from environment any time	3 (27.3%)	15 (23.4%)	0.719
Number (%) where <i>C difficile</i> was grown from environment more than three weeks after treatment for diarrhea	3 (27.3%)	4 (6.8%)	0.060
Number (%) who were prolonged excretors	9 (81.8%)	9 (14.1%)	<0.0001 relative risk 14.25 (3.383-60.023)

*Mann-Whitney Test for nonparametric data, Two-sided P value

13.88) to have received antibiotics other than metronidazole and/or vancomycin at the time of, or just before, their recurrence of diarrhea (Figure 2) compared with patients who did not have diarrhea recurrence (22 of 64, 34.4%). Cephalosporins were the 'other concurrent' antibiotics most commonly used, but this was not significantly greater than the nondiarrhea recurrence group.

The relative risk of prolonged excretion was 14.25-fold greater (95% CI 3.38 to 60.02) for diarrhea recurrence compared with nondiarrhea recurrence patients (Table 1). Prolonged excretion was defined as the presence of toxigenic *C difficile* and/or detectable *C difficile* toxin(s) at week 3 or later after antibiotic therapy was initiated for CAD. This three-week postinitiation of antibiotic therapy cutoff was chosen because it was expected to represent one week after the completion of antibiotic therapy for CAD (protocols for metronidazole or vancomycin to treat CAD most commonly last 10 to 14 days). Fifty-one stool samples from the 75 patients were positive (beyond week 1) by at least one of the three test methods (cytotoxin testing, culture or ELISA). Of these 51 samples, the cytotoxin assay was positive in 44 of 51 (86.3%), ELISA in 30 of 51 (58.8%) and CCFB enrichment culture in 20 of 51 (39.2%). Prolonged excretion of *C difficile* toxin(s) or toxigenic organism for three weeks or more occurred in 18 of the 75 patients enrolled (Figure 3). Only one patient had multiple diarrhea recurrences. This patient had detectable toxigenic-*C difficile* in five of the nine available stool samples over 13 weeks of hospitalization. Although diarrhea recurrence correlated with prolonged excretion, most instances of diarrhea recurrence occurred in the first three to four weeks after the diagnosis of CAD. In many instances, a toxigenic organism did not grow despite a positive cytotoxin or ELISA test (Figure 3).

When the isolation of toxigenic *C difficile* environmental isolates was stratified by week of hospitalization, 12 of 13 (92.3%) of the environmental contaminants detected from week 3 onwards came from rooms of five patients who were prolonged excretors. The maximum length of continued environmental contamination for any one patient was four weeks. In most instances (13 of 16), environmental contamination was detected only once. It was unlikely that the environmental contamination with toxigenic *C difficile* was due to high endemic levels of environmental contamination, because environmental samples obtained in week 1 of hospitalization indicated that patients with CAD had significantly ($P=0.002$) more environmental contamination (14 of 75, 18.7%) compared with patients with non-CAD (two of 75, 2.6%). The relative risk was 1.92 (95% CI 1.47 to 2.49). The bed pan sprayer (CAD eight, non-CAD two) was the most common site followed by the toilet (CAD five, non-CAD zero) and the bed rail (CAD one, non-CAD zero).

Fifteen patients with CAD had at least one toxigenic *C difficile* isolate available for PFGE. Isolates from stool came from week 2 onwards. Environmental cultures for toxigenic *C difficile* were performed every week. There were, from all sources, 60 isolates of toxigenic *C difficile* available for PFGE. Of the 57 that had discernable banding patterns that allowed compara-

Figure 2) Antibiotic therapy in patients who had recurrence of diarrhea. Patients with *Clostridium difficile*-associated diarrhea were prospectively followed for as long as the patient was in hospital (up to a maximum duration of 13 weeks). Although the patients were admitted at different times, the time line is given as days post enrollment in the study (ie, initial occurrence of diarrhea that was positive for *C difficile* toxin). The patients with a recurrence of diarrhea after appropriate therapy were graphed to correlate antibiotic therapy with time of diarrhea recurrence. The patients who were nonprolonged excretors have been identified. Therapy with vancomycin and metronidazole have been recorded on the top line for each patient, and 'other antibiotic' therapy has been recorded on the middle line. The lines that have arrows for antibiotic therapy indicate the stop date for the antibiotic(s) was not indicated on the patient's chart. Duration of diarrhea has been indicated on the bottom line for each patient. The number given just before the recurrence of diarrhea indicates the number of days that elapsed after the completion of the FIRST course of metronidazole and/or vancomycin (given for the enrolling bout of diarrhea) until the diarrhea recurrence started.

tive analysis, 26 were from stool samples, 21 were from bedpan sprayers, six were from toilets, one was from a commode, two were from bedrails and one was from a buzzer. Among these 57 isolates, there were 21 different PFGE profiles (at least seven or more band differences between each PFGE profile). The 26 stool isolates came from 15 different patients, and, among these patients, there were 14 different PFGE profiles (Figure 4). Thus, one endemic strain did not appear to account for all CAD in the hospital. However, for the five patients with both a stool and environmental isolate available for analysis, the PFGE profile of at least one of the isolates detected in the environment matched that of the stool isolate in

Figure 3) Excretion pattern in patients treated with antibiotics for *Clostridium difficile*-associated diarrhea. Of the 75 patients with CAD who were enrolled in the study, 18 (24%) were prolonged excretors. Stool samples were assayed each week and were recorded as negative, ELISA positive only, cytotoxin B positive only, and cytotoxin B as well as ELISA positive. The presence of culture-confirmed *C. difficile* that was toxigenic is shown by (*). Some weeks there was no stool sample available for evaluation and this was indicated by ("N"). The week of diarrhea recurrence have been denoted by an arrow (➔). When no further symbols are recorded, this indicates that the patient was discharged from the study

four instances. Because isolates were not obtained from the stool in week 1, it was not possible to determine whether patients excreted or had a diarrhea recurrence due to the same strain that caused their original disease. However, of the six patients with multiple stool isolates from different dates available for analysis, four had prolonged carriage of isolates that had the same PFGE profile. The maximum length of carriage of the same PFGE profile organism was three months. Although, it was not specifically assessed as to whether one stool sample may harbour multiple strains with different PFGE profiles, four patients had isolates from stool samples taken on different days that had different PFGE profiles, indicating that colonization may be transient.

DISCUSSION

Although asymptomatic carriage of *C. difficile* in patients without diarrhea has been described, little is known about excretion of the organism from patients whose initial CAD has been appropriately treated with antibiotics. Our study results demonstrated that patients with CAD who had a diarrhea recurrence despite appropriate antibiotic therapy were at significantly greater risk of being prolonged excretors ($P < 0.0001$, relative risk 14.25; 95% CI 3.38 to 60.02). To our knowledge, this is the first published study to indicate that prolonged excretion may have clinical relevance. These prolonged excretors represent a different population from that described by Johnson et al (16) who reported that asymptomatic carriers (who did not have prior diarrhea) were not at increased risk of developing disease. The balance of normal bowel flora in asymp-

Figure 4) Pulsed-field gel electrophoresis profiles (PFGE). From the 57 isolates available for analysis, there were 21 distinct PFGE profiles. This micrograph shows six of the 21 PFGE profiles. Lane 1 contains the PFGE markers, lanes 2 to 15 contain samples of *Clostridium difficile* DNA digested with *Sma* I. Lanes 7, 9, 10, 11 contain isolates that could not be analyzed because the DNA was degraded and did not produce banding patterns (despite several attempts). The other samples included stool isolate patient 29 (lane 2); stool isolate patient 32 (lane 3); bed pan sprayer isolate patient 35 (lane 4); stool isolate patient 41 (lane 5); stool isolate patient 41 (lane 6); stool isolate patient 55 (lane 8); stool isolate, patient 52 (lane 12); sprayer isolate patient 52 (lane 13); stool isolate patient 52 (lane 14); and stool isolate patient 52 (lane 15)

tomatic patients may be quite different from the flora of those patients who have had CAD therapy. The risk of diarrhea recurrence in our post-therapy prolonged excretors may be because the surviving *C. difficile* could readily overgrow the normal bowel organisms that may still be out of balance due to antibiotic pressure. Although 15% of the original patients developed diarrhea recurrence, the true incidence cannot be reliably established in this study because many CAD-treated patients were discharged within one week of therapy initiation and were not available for further follow-up. Despite this limitation, the correlation between diarrhea recurrence and prolonged excretion warrants further assessment to determine the clinical relevance of prolonged excretion. Patients with diarrhea recurrence were significantly more likely to have received antibiotics (nine of 11 [81.8%]), other than metronidazole and/or vancomycin, just before or within 30 days of developing diarrhea recurrence than patients who had no diarrhea recurrence (22 of 64 [34.3%]). This raises the possibility that diarrhea recurrence was linked to the use of concurrent 'other' antibiotics and highlights the significance of discontinuing other antibiotics where possible when patients are being treated for CAD. Although the discontinuation of the 'offending' original antibiotic that precipitated the CAD has always been recommended (22), another antibiotic may be necessary to treat the initial underlying infection.

Patients with CAD are placed on some form of isolation pre-

cautions (eg, enteric precautions) in most hospitals (22). How to determine when such infection control precautions can safely be discontinued has not been well defined (22). Wilcox and Spencer (17) found that "stool culture or toxin positivity is recognized to occur commonly following antibiotic therapy for CAD, in patients who nevertheless remain asymptomatic". Although not recommended (22), post-therapy tests are sometimes done because caregivers are not sure when enteric precautions can be discontinued for patients who have been appropriately treated and are now asymptomatic. This often creates a dilemma about what to do if the patient no longer has diarrhea but has a positive diagnostic test for toxigenic *C difficile*. Such 'continued excretion' in patients treated for CAD has not been well studied. Although our data indicate a correlation between diarrhea recurrence and prolonged excretion, further studies are warranted to confirm our finding that there may be clinical significance to prolonged excretion post CAD-therapy, and to determine whether there is a way to identify reliably patients who are most likely to have a recurrence of their diarrhea. Because most diarrhea recurrence occurs within three weeks of the initiation of CAD therapy, it might be prudent to ensure enteric precautions for at least three weeks post-CAD diagnosis and then discontinue enteric precautions for patients still in hospital who no longer have diarrhea.

Our study demonstrated that environmental contamination at the time of diagnosis of CAD (week 1) was significantly more common in patients with this disease than in patients with diarrhea due to other causes (18.6% versus 2.6%). Additionally, there was a subset of patients in the CAD group (six of 75) who went on to have prolonged environmental contamination (for three weeks or more). The role of environmental contamination during the acute diarrheal phase or the asymptomatic prolonged excretion phase in nosocomial disease transmission is not known (22). Isolates from week 1 were not available for typing, and only one colony of *C difficile* was taken per environmental site, so we cannot determine whether the prolonged environmental contamination was due to the same strain that originally caused disease in the patient. However, despite these caveats, our data clearly demonstrate that there is significantly greater risk of having toxigenic *C difficile* in the environment of individuals with CAD compared with those with diarrhea from other causes. This is in agreement with the observation that those patients who develop CAD often have contact with another person carrying the organism, and that progression to disease (if it occurs) of the newly admitted patient usually occurs shortly thereafter (23). The patient with CAD and the antibiotic-treated prolonged excretors may provide a source of toxigenic *C difficile* for patients who acquire the organism exogenously.

Studies that assessed the strains involved in diarrhea recurrence indicated that approximately 50% of *C difficile* isolates obtained from 'relapsing' patients were due to strains different from the initial infecting strain when assessed by restriction enzyme analysis, suggesting they actually represented reinfection (19,20). However, there was no attempt to determine whether the patients were prolonged excretors. The lack

of strains available for PFGE limited our ability to assess reliably whether diarrhea recurrence was due to the same or a different strain.

CONCLUSIONS

Our study demonstrated a correlation between development of diarrhea recurrence and prolonged excretion of cytotoxin, as well as the use of concurrent antibiotics for other underlying infections. The need to discontinue antibiotics when CAD is diagnosed is supported by our study because failure to do so may predispose the patient to developing a diarrhea recurrence. Additional antibiotics should be prescribed only if they are still indicated to treat the underlying infection. In addition, every attempt should be made to ensure that the physician is dealing with a true infection and not a colonization when additional antibiotic administration is being considered. Further studies are needed to determine whether there is a way to predict reliably who will develop diarrhea recurrence and to determine whether any beneficial interventions can be identified.

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