Yersinia infection with *Clostridium difficile* colitis

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ABSTRACT: Antibiotic associated diarrhea appears to be largely due to *Clostridium difficile* and may have, at least in part, a toxin mediated pathogenesis. Because a poor correlation exists between measurable toxin titres and symptoms, additional copathogenic factors may be important. All patients seen during a two year period with diarrhea within four weeks of antibiotic therapy, a positive stool culture for *C difficile* and a positive stool cytotoxin assay specific for *C difficile* were investigated. All patients had stools cultured for enteric bacterial pathogens including *Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas* and *Yersinia* species. Seven patients had *Yersinia* species isolated during the course of their illness; no other enteric pathogens were identified. In four patients, *Y enterocolitica* was cultured simultaneously with *C difficile* prior to treatment, and in one of these, *Y fredriksenii* was also isolated. Of six patients with persistent or recurrent symptoms after treatment for *C difficile* (ie, vancomycin in five and metronidazole in one patient), four had positive yersinia cultures at the conclusion of therapy (*Y enterocolitica* in three and *Y fredriksenii* in two patients). All but one of these patients had been yersinia culture negative prior to therapy for *C difficile*. Patients with and without yersinia isolates were then compared with respect to age, sex, clinical symptoms and sigmoidoscopic as well as rectal biopsy findings. The presence of yersinia was associated with male sex, younger age and abdominal pain; other features including hematochezia, fever, arthralgia, cytotoxin titre, sigmoidoscopy and rectal biopsy could not distinguish patients with and without *Yersinia* species. Thus, yersinia may be associated with an antibiotic related diarrheal illness usually attributed to *C difficile* alone and may be observed in the setting of persistent or recurrent symptoms following treatment for *C difficile* diarrhea. **Can J Gastroenterol** 1989;3(1):21-25

Key Words: Antibiotic diarrhea and colitis, *Clostridium difficile*, *Clostridium difficile* cytotoxin, Pseudomembranous colitis, *Yersinia* diarrhea, *Yersinia enterocolitica*

L'infection à yersinia et la colite attribuable à *Clostridium difficile*

RESUME: Les diarrhées associées aux antibiothérapies semblent surtout attribuables à *Clostridium difficile* et pourraient présenter, en partie tout du moins, une pathogenèse liée aux toxines. Parce qu'il existe une faible corrélation entre les titres de toxines mesurables et les symptômes, les facteurs co-pathogènes supplémentaires sont peut-être importants. Ont été examinés: tous les patients (14) vus sur une période de deux ans et souffrant de diarrhées moins de quatre semaines après une antibiothérapie, et ayant subi un coproculture destinée à repérer *C difficile* avec résultats positifs. Pour

COLITIS MAY COMPLICATE ANTIMicrobial therapy and, in the vast majority of patients, appears to be due to *Clostridium difficile* (1). In most cases, treatment with oral vancomycin is effective although one or more symptomatic relapses may occur (1). Failure to eradicate the organism, possibly due to sporulation, or acquisition of a new strain of *C difficile*, may be responsible (2). Alternatively, coinfection with another organism or acquisition of a second organism could occur.

In the present study, all patients seen in the hospital during a two year period with *C difficile* associated colitis were evaluated prior to and at the conclusion of therapy for a second bacterial pathogen or parasite. In this specific setting, a significant proportion of patients with disease initially attributed to *C difficile* were observed to have *Yersinia* species infection, but no other, bacterial pathogens.

MATERIALS AND METHODS

Specimen source: Fecal specimens were derived from inpatients being investigated for diarrhea or from patients referred to an outpatient clinic specializing in gastrointestinal diseases; a small number of specimens were submitted from a university student health service. During the two year period of this study, 403 stool specimens from 269 patients were cultured for *C difficile* and its cytotoxin; all stool specimens were routinely cultured for *Yersinia* species. A total of 14 patients were identified with diarrhea beginning within four weeks of antibiotic administration associated with a positive stool culture and cytotoxin assay for

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tous les patients, la mise en culture bactériologique a porté sur les agents pathogènes entériques — *Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas* et *Yersinia* species y compris. Durant le cours de leur maladie, sept des patients étaient porteurs des espèces *Yersinia* et aucun autre agent pathogène n'a été identifié. Pour quatre patients, *Y enterocolitica* et *C difficile* ont été cultivés simultanément avant le traitement, et chez l'un d'eux, *Y fredriksonii* a été isolé. Chez six patients souffrant de symptômes persistents ou récurrents après avoir été traités pour *C difficile* (vancomycine chez cinq des patients et métronidazole pour l'autre), quatre ont donné des cultures positives pour la détection de *Yersinia* au terme de la thérapie (*Y enterocolitica* chez trois d'entre eux et *Y fredriksonii* chez les deux autres). À l'exception d'un seul, tous ces patients avaient donné des résultats négatifs avant d'être traités contre *C difficile*. Les patients chez qui *Yersinia* avait été isolé ou non, ont ensuite été comparés selon l'âge, le sexe, les symptômes cliniques et sigmoïdoscopiques, ainsi que d'après les résultats des biopsies rectales. La présence de *Yersinia* est associée aux patients de sexe masculin, d'un âge relativement plus jeune et souffrant de douleurs abdominales. Les autres caractéristiques incluent l'émission de selles sanglantes, la fièvre, l'arthralgie. Les titres de cytotoxines, les sigmoïdoscopies et biopsies rectales ne sont parvenues à établir aucune distinction entre les malades atteints de divers types de *Yersinia* et les autres. Ainsi, *Yersinia* peut être associé aux diarrhées consécutives aux antibiothérapies que l'on a coutume d'attribuer à *C difficile* seulement et peut être détecté lors des symptômes persistants et récurrents qui se manifestent après le traitement des diarrhées provoquées par *C difficile*. La colite qui complique parfois les thérapies antimicrobiennes semble attribuable, dans la grande majorité des cas, à *C difficile*. Le plus souvent, un traitement à base de vancomycine par voie orale suffit, bien qu'une rechute symptomatique ou plusieurs puissent se produire. Si l'on ne parvient pas à éliminer l'organisme, il est possible qu'il y ait sporulation ou qu'une nouvelle souche de *C difficile* soit responsable de l'échec; ou encore, qu'il y ait co-infection par un autre organisme ou acquisition d'un second agent. Dans l'étude présente, qui porte sur une période de deux ans, tous les patients atteints d'une colite associée à *C difficile* ont été évalués avant et au terme d'une thérapie destinée à combattre un second agent pathogène ou parasite. Dans ce cadre particulier, un nombre significatif des patients atteints de maladies tout d'abord attribuées à *C difficile* se sont avérés porteurs de *Yersinia*, mais d'aucun autre agent bactérien pathogène.

C difficile. Of these, no patient developed diarrhea in hospital and all were referred from within British Columbia. Stool specimens were obtained from all patients at the onset and conclusion of therapy for diarrhea presumed to be caused by *C difficile* infection. Specimens were submitted in containers with Cary-Blair transport medium or fresh without transport medium.

Culture media and processing: All specimens were routinely cultured on sheep blood agar (Prepared Media Laboratories, Tualatin, Oregon), McConkey agar (Difco, Detroit, Michigan), deoxycholate agar (Difco), thiosulphate-citrate-bile-sucrose agar (Oxoid, Basingstoke, UK), campylobacter agar (PML, Tualatin, Oregon), aeromonas agar (Difco) and selenite broth (Oxoid). Isolates of *Escherichia coli* were tested for their ability to ferment sorbitol as a screen for hemorrhagic colitis associated strains. Approximately 90% of the specimens were ac-

companied by additional fecal samples to examine for the presence of intestinal parasites. No fecal samples were examined for enteric viruses.

Yersinia culture and detection methods: Fecal material was streaked on yersinia selective (Schiemann CIN) medium (Gibco, Madison, Wisconsin) and incubated at 35°C for 18 to 24 h after cold enrichment intervals of 24 h, three days and again after one and two weeks.

Yersinia species were first detected by their characteristic colonial morphology appearance on yersinia selection CIN medium. As described elsewhere (3),

typical colonies have a 'bullseye' appearance with a deep cherry red centre and transparent margins. Colonies also have a ground glass appearance under the stereomicroscope and have a distinctive odour. Suspect yersinia isolates were further screened using a routine triple sugar iron agar slant for detection of glucose, lactose and sucrose fermentation or hydrogen gas production, as well as an urea slant for urease production. In addition, lysine and indole test reactions as well as motility after incubation at 28°C were assessed. Further confirmation of *Yersinia* series was done using an API 20E enterobacteriaceae system (API Laboratory Products Inc, St Laurent, Quebec), incubated at 28°C. A typical group of sugar fermentation reactions for yersinia permitting initial distinction of the different species is tabulated in Table 1. All positive isolates identified to species were submitted to an independent reference laboratory for confirmation as well as definition of biotype and serotype of the organism.

Clostridium difficile: *C difficile* was cultured anaerobically on CD and Columbia CNA media for 48 h. (Capco Anaerobic Environmental System, Santa Clara, California). Identification was based on colonial appearance, typical 'barnyard odour', Gram stain morphology, aerobic and anaerobic subculture and anaerobic API sugar fermentation profile. Cytotoxin assay followed the method of Allen (4) using filtered stool supernatant (10,000 g; 0.45 µm filter pore size), human foreskin fibroblast cultures and *C difficile* antitoxin (TD Wilkins, Virginia Polytechnic Institute, Blacksburg, Virginia). Titres were determined using serial 10-fold dilutions of the supernatant.

RESULTS

Clinical features: Table 2 shows the age and sex of the 14 patients with *C difficile*

TABLE 1
Typical sugar fermentation reactions of *Yersinia* species

Species	Sucrose	Rhamnose	Raffinose	Melibiose
<i>Y enterocolitica</i>	+	—	—	—
<i>Y fredriksonii</i>	+	+	—	—
<i>Y intermedia</i>	+	+	+	+
<i>Y kristensenii</i>	—	—	—	—

TABLE 2
Clinical features

Clinical feature	Negative	Yersinia cultures Positive (before therapy)	Positive (after therapy)
Age (years)			
Range	24-89	31-77	27-77
Mean	61	48	52
Sex			
Male	2	2	2
Female	5	2	3
Diarrhea (bloody)	7 (1)	4 (1)	5 (1)
Abdominal pain	2	3	2
Fever	2	1	1
Arthralgias	1	0	1

diarrhea. *Yersinia* species tended to occur in younger patients compared to the group with negative cultures. In addition, males were more often likely to have a positive culture.

Table 2 also shows symptoms elicited in the 14 patients with *C difficile* diarrhea. Inclusion criteria required the presence of diarrhea within four weeks of antibiotic therapy; one patient with bloody diarrhea had a negative yersinia culture, one had a positive initial culture and one with initially negative cultures, whose diarrhea became bloody after failed treatment, subsequently had yersinia isolated.

Abdominal pain or tenderness at the time of initial culture was more common when yersinia was present (three of four patients, 75%) than when yersinia was absent (two of seven patients, 29%). With failed therapy for *C difficile*, one patient had persistent abdominal pain and persistence of *Y enterocolitica* in stool. In one patient with persistent diarrhea and initially negative cultures for yersinia, abdominal pain and the presence of both *Y enterocolitica* and *Y fredrikseii* developed after vancomycin therapy for *C difficile*. Duration of symptoms did not differ in yersinia positive patients compared to yersinia negative patients.

Neither fever nor arthralgia could predict the presence or absence of *Yersinia* species. Fever was present in two patients with negative yersinia cultures, in one patient with a positive *Y enterocolitica* isolate who responded well to vancomycin therapy and in one patient who had recurrent diarrhea with *Y enterocolitica* and *Y fredrikseii* isolates after being initially yersinia culture negative.

Stool cultures: Results of yersinia cultures are shown in Table 3. All 14 patients had a positive stool culture for *C difficile* simultaneously with positive assay for *C difficile* cytotoxin. Four patients (29%) also had confirmed isolation of *Y enterocolitica* from the same initial stool specimen while *Y fredrikseii* was concomitantly identified in one of these patients. In all patients, positive cultures for *Yersinia* species were not confirmed for 14 days; therefore, therapy initiated to eradicate *C difficile* diarrhea was not altered because of subsequent detection of *Yersinia* species.

Six of 14 patients (43%) had persistent or recurrent diarrhea following treatment with vancomycin (five patients) or metronidazole (one patient). Further stool cultures after this therapy resulted in isolation of *Yersinia* species in four of these six patients (67%): *Y enterocolitica*

in three patients and *Y fredrikseii* in two (isolated concurrently with *Y enterocolitica* in one patient). Only one patient with *Y enterocolitica* had been yersinia culture positive prior to treatment. One further patient with a positive *Y enterocolitica* culture at the onset of treatment for diarrhea became asymptomatic after vancomycin therapy. However, a repeat stool culture after 16 days of treatment revealed *Y intermedia*; in this patient neither *C difficile* nor *Y enterocolitica* could be identified following treatment. No other enteric pathogens were isolated at any time during the illnesses of these 14 patients.

Sigmoidoscopic and biopsy findings: Flexible fiberoptic sigmoidoscopy with biopsy was done in all 14 patients at the onset of symptoms and was repeated in all patients with failed therapy (Table 4). The endoscopic and histologic observations did not differentiate patients with yersinia isolated in stool cultures compared to patients without positive cultures. The patient with persisting *Y enterocolitica* before and after treatment has a 'nonspecific' colitis histologically confirmed at both times with positive cultures.

Clostridium difficile cytotoxin titres: Table 5 shows *C difficile* cytotoxin titres in patients with and without positive yersinia isolates. No differences in toxin titres were observed. All four patients with persistent diarrhea and positive yersinia cultures after therapy had *C difficile* cytotoxin titres measured; all of these patients had negative cytotoxin assays after therapy. Two other patients with persistent symptoms following treatment had negative yersinia cultures; one of these patients had a persistently positive *C difficile* stool cytotoxin assay (titres of 1/10,000 before and after a course of vancomycin therapy).

DISCUSSION

Several organisms have been etiologically implicated in post antibiotic colitis. *Staphylococcus aureus* received much attention initially (5) and, more recently, *C perfringens* has been suggested as a possible cause (6). However, the majority of cases are presently ascribed to *C difficile*. *C difficile* produces an enterotoxin (ie, toxin A) and a potent cytotoxin (ie, toxin

TABLE 3
Yersinia cultures in Clostridium difficile diarrhea

Species	Culture positive before treatment	Culture positive after treatment
<i>Y enterocolitica</i>	4	3
Biotype 1, Serotype O:41	1	0
Biotype 1, Serotype O:6,30	2	1
Nontypeable	2	2
<i>Y fredrikseii</i>	1	2
<i>Y intermedia</i>	0	1

TABLE 4
Sigmoidoscopic and biopsy findings

Pathology features	Negative	Yersinia cultures Positive (before therapy)	Positive (after therapy)
Performed	7	4	4
Pseudomembranous colitis	3	1	2
Aphthoid ulceration	1	0	1
Nonspecific diffuse colitis	2	2	1
Normal	1	1	0

TABLE 5
***Clostridium difficile* cytotoxin titres**

Titre	Negative	Yersinia cultures Positive (before therapy)	Positive (after therapy)
1/100	3	2	4
1/1000	3	0	0
1/10,000	1	2	1

B) (7-9). The cytotoxin may induce typical changes in the colonic mucosa (8) and its detection has been used to establish the diagnosis (1). However, there appears to be a poor correlation between cytotoxin titre (toxin B) and the severity of the clinical illness (10). Although toxin A may correlate more strongly with symptoms, other factors may also be involved (11).

Of 108 patients with post antibiotic *C difficile* colitis in Sweden, none had other enteric pathogens isolated; some patients, however, had detectable cytotoxin but negative cultures for *C difficile* (12). This raised the possibility of *C difficile* cytotoxin production by undetected organisms, perhaps other than *C difficile* (12, 13). Bartlett (14) has reported that five of 144 patients with significant titres of *C difficile* cytotoxin had negative cultures; attempts to identify other organisms in such circumstances were unsuccessful. In contrast, other studies have indicated that more 'traditional' pathogens such as *Salmonella* and *Campylobacter* species may be isolated from patients with *C difficile*. Culture techniques and/or, possibly, geographic factors may have accounted for the unusually high frequency of yersinia in seven (or 50%) of the present patients with *C difficile* diarrhea. Post antibiotic colitis due to *Y enterocolitica* in the absence of *C difficile* has also been reported (17). Finally, three

patients with *Y enterocolitica* associated diarrhea have been described with *C difficile* cytotoxin also detected (3).

Administration of antibiotics in the month prior to positive stool culture was previously noted in 29 of 122 patients with *Y enterocolitica* associated diarrhea (3). The role of antibiotics in predisposing to coinfection with *Yersinia* species and *C difficile* is uncertain but compelling. Of particular interest is the role of vancomycin or metronidazole therapy in the development of new isolates of *Yersinia* species in four patients reported here. This seemed to be especially associated with persisting or recurrent diarrhea.

Clinical differentiation of patients with and without yersinia in the presence of *C difficile* appears to be difficult. Abdominal pain was more common with yersinia in the present study, but this symptom is well recognized in patients with *C difficile* colitis (18). Fever and bloody diarrhea were also present in both groups. Arthralgias, reported in up to 30% of patients with *Y enterocolitica* infection (19,20) also occurs in *C difficile* colitis (21-24) and could not predict the presence of *Yersinia* species in the present patients.

Yersinia enterocolitis has been associated with normal mucosa, nonspecific colitis and colonic mucosal aphthoid ulcerations (25), but in this study, sig-

moidoscopic examination and rectal mucosal biopsy of patients with *C difficile* diarrhea could not differentiate between patients with and without yersinia coinfection. Pseudomembranes were seen in a minority of patients with and without yersinia. Two of the present patients, both having isolates of *C difficile* and *Y enterocolitica*, presented as an exacerbation of previously diagnosed inflammatory bowel disease; both *C difficile* (26-28) and yersinia (3,29) have been implicated in this setting. Under these circumstances, morphologic diagnosis may be very difficult.

In summary, an inordinately high prevalence of *Yersinia* species exists in the stools of patients referred to the authors' institution with post antibiotic *C difficile* colitis. Failed therapy of *C difficile* colitis may be associated with yersinia coinfection. *Yersinia* species, particularly *Y enterocolitica*, must be considered potential enteric pathogens in the post antibiotic state and the confirmation of *C difficile* does not obviate the need to carefully seek their presence in this situation.

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