**Clostridium difficile** and inflammatory bowel disease

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**Summary**

Stools from 109 patients with inflammatory bowel disease (13·4%) contained *Clostridium difficile* or its toxin, an incidence similar to the stools of 99 control patients with diarrhoea (11·9%), but significantly higher than the stools of 77 control patients with a normal bowel habit (1·4%). Sixty-six per cent of the diarrhoea controls, but only 11% of the inflammatory bowel disease patients, reported recent antibiotic use: however, 67% of inflammatory bowel disease patients were taking sulphasalazine. The presence of *Cl difficile* in the stool was not related to the clinical assessment of inflammatory bowel disease relapse, but it was related to hospital admission. During the one year study, 31 of the 109 patients (28%) with inflammatory bowel disease had one or more stool samples that were positive for *Cl difficile*.

Recent studies have shown that a toxin of *Clostridium difficile* has a pathogenic role in patients with pseudomembranous colitis or antibiotic associated diarrhoea without pseudomembrane formation.1-3 Pseudomembranous colitis may occur without any predisposing factor;4 but there is some evidence that *Cl difficile* may be infectious.5-8

There are five reports9-13 concerning the identification of *Cl difficile* or its toxin in stools of patients with inflammatory bowel disease: three reports conclude that *Cl difficile* is found frequently in such patients, especially when there is active inflammation.9-11

In the present study, stool samples from patients with inflammatory bowel disease have been compared with samples from two groups of control patients. The presence of *Cl difficile* or its toxin in a patient’s stool has been correlated with recent exposure to antibiotics or sulphasalazine, activity of inflammatory bowel disease, and hospital admission.

**Methods**

**Patients with inflammatory bowel disease**

From October 1980 to September 1981 67 patients with ulcerative colitis and 42 patients with Crohn’s disease took part in the prospective study. The diagnosis of inflammatory bowel disease was based upon accepted clinical, radiological, and histological criteria.

Whenever a patient was seen as an outpatient, or approximately every week on the ward, a record was made of frequency of bowel actions, the patient’s well-being (better, same, worse), the duration of clinical relapse, the presence of blood or mucus in the stool, and the drug therapy (including antibiotic use) during the preceding month. Physical examination included sigmoidoscopy. On the basis of this information, the physician assessed whether the patient’s inflammatory bowel disease was in remission or relapse, and then treated the patient appropriately using a conventional combination of steroids, sulphasalazine, and occasionally azathioprine. No disease activity index was used. Blood samples were taken for haemoglobin, white count, ESR, and serum albumin. The clinicians were not informed of the stool analysis results for either *Cl difficile* or its toxin until the end of the study.

**Control patients with diarrhoea**

One hundred and twenty-eight stool samples were obtained from 99 hospital inpatients with acute diarrhoea. Sixty-six per cent of these stool samples came from patients with a recent exposure to antibiotics. These clinical samples had been sent to the laboratory for routine *Cl difficile* analysis, as either pseudomembranous colitis or antibiotic associated diarrhoea was suspected.
HEALTHY CONTROL PATIENTS
Seventy-seven stool samples came from hospital
outpatients, who had attended the hospital for
upper gastrointestinal endoscopy. All except one
patient reported either normal bowel habit or
constipation when these stool samples were
collected. Seven samples were excluded: six patients
had taken antibiotics in the preceding month and
one had recently been an inpatient, and was
receiving large doses of cimetidine, chlorphenir-
amine maleate, and hydroxyurea for systemic
mastocytosis.

CLOSTRIDIUM DIFFICILE CULTURE
A selective medium (Oxoid Ltd, Basingstoke,
Hampshire) consisting of an agar base, a selective
supplement (containing D-cycloserine and cefoxitin)
and 7% defibrinated horse blood (Gibco, Europe)
was used for the culture of Cl difficile from the stool
samples. A light inoculum of the stool was spread
over each plate and incubated in a Coy anaerobic
chamber at 37°C for 48 hours. Plates were inspected
under ultraviolet light and colonies showing a typical
appearance were subcultured to obtain a pure
growth of the organism. Identity of Cl difficile was
confirmed by demonstration of characteristic
patterns of fermentation of 1% carbohydrates
incorporated in Fastidious Anaerobic Broth
(London Analytical & Bacteriological Media Ltd,
Salford, Manchester M6 6PB).

CLOSTRIDIUM DIFFICILE TOXIN ASSAY
Ringer's solution was added to stools for toxin assay
to give a 1-in-4 dilution of liquid stools, and a 1-in-8
dilution of solid stools. The suspension was then
centrifuged at 3000 g for 10 minutes, followed by
15 000 g for 20 minutes and the supernatant passed
through a 0-2 μm membrane filter (Microflow 25,
Flow Laboratories, Irvine, Scotland). Monolayers of
MRC5 fibroblasts were prepared in microtitre
wells (Sterilin Ltd, Teddington, Middlesex). Stool
filtrates were diluted 1-in-10 in tissue culture medium
(Eagle's minimum essential medium with 2% fetal calf serum) and 0-1
ml of the dilution added to each of two microtitre
wells. One well contained 0-1 ml of a 1-in-10 dilution
of Cl sordellii antitoxin (Wellcome Research
Laboratories, Beckenham, Kent) in tissue culture
medium and the other contained 0-1 ml of tissue
culture medium alone. The monolayers were
examined for a cytopathic effect after five, 24, and
48 hours' incubation at 37°C. A clear, typical
cytopathic effect in the well containing the stool
filtrate alone, but not in its sister well which also
contained antitoxin, was recorded as a positive toxin
assay.

STATISTICAL ANALYSIS
χ² test, with no correction for continuity.

Results
During the year's study, 333 stool specimens were
collected from patients with inflammatory bowel
disease, an average of 3-1 specimens per patient
(range 1-14). Patients were unable to provide a
stool sample on 62 occasions. Approximately equal
numbers of specimens were obtained from patients
with ulcerative colitis during periods of remission
and relapse (110 and 108 respectively), but more
specimens were obtained from patients with Crohn's
disease during the remission than relapse (71 and 43,
respectively).

Table 1 shows that Cl difficile and/or its toxin were
found in 13-2% of stool samples from patients with
Crohn's disease, in 13-7% of samples from patients
with ulcerative colitis, and in 11-7% of samples from
the inpatient controls with diarrhoea. As either
culture of Cl difficile or detection of its toxin signifies
the presence of the organism in a stool sample, the
detection of either marker identified a stool sample
as 'Cl difficile positive'. Only one sample from the
healthy outpatient controls was positive for Cl
difficile (1-3%). In all three (Crohn's disease, ulcerative
colitis, and diarrhoea) groups, positive
samples were significantly more frequent than in the
healthy outpatient controls (p<0-01).

Table 2 shows that the incidence of Cl difficile
positive stool samples was virtually identical during either clinical remission or relapse of inflammatory bowel disease (14% and 13%, respectively). Thirteen per cent of stool samples from patients taking only sulphasalazine were positive, but the history of antibiotic use during the preceding month significantly increased the incidence of positive samples to 31% (p<0.01). Antibiotics used by the patients were: amoxycillin, cefuroxime, dapsone, erythromycin, fluoroaxillin, metronidazole, mezlocillin, rifampicin, co-trimoxazole, and oxytetacycline. Patients taking neither sulphasalazine nor antibiotics had 5.5% positive stools: although this was considerably lower than the incidence found in patients taking sulphasalazine alone, the difference is not statistically significant (p<0.1). Fifty-two per cent of the patients not taking either sulphasalazine or antibiotics were in remission, compared with 45% of the remaining inflammatory bowel disease patients: in neither subgroup did Clostridium difficile positive stool samples relate to disease activity.

The isolation of Clostridium difficile or its toxin from the stools was significantly related to hospital admission (p<0.01). Twenty-four patients with inflammatory bowel disease were admitted to hospital during the year, 12 had one or more positive stools for Clostridium difficile. The other 19 patients with positive stools had no history of hospital admission during the study year. Of the patients admitted to hospital, four had a total colectomy, two had a hemicolectomy with a resection of the terminal ileum, and one died with colonic perforation. Five of the six patients requiring surgery had Clostridium difficile in their stool at some time, but these patients had all been exposed to recent antibiotics. There was no histological evidence of pseudomembranous colitis in the resected colonic material.

Discussion

Clostridium difficile is found frequently in the stools of patients with inflammatory bowel disease, without exposure to broad spectrum antibiotics. In the present study 13.4% of stools of patients with inflammatory bowel disease were Clostridium difficile positive, a result very similar to the 11.9% positive stools from one of this study’s control groups (hospital inpatients with diarrhoea) and a study of diarrhoea after antibiotic treatment.16 The main difference between the two groups in this study was that whereas 66% of the diarrhoea control stools had been exposed recently to antibiotics, only 11% of the samples from the inflammatory bowel disease had been so exposed (p<0.01). Sixty-seven per cent of the inflammatory bowel disease stools, however, had been exposed to sulphasalazine, which is deconjugated in the colon, releasing free sulphapyridine in the stools.17

The patients with inflammatory bowel disease with a history of recent antibiotic exposure had a significantly higher incidence of Clostridium difficile in their stools than those taking only sulphasalazine (31% against 13%, respectively, p<0.01). Stools from patients with inflammatory bowel disease taking neither antibiotics nor sulphasalazine, however, had an even lower incidence of Clostridium difficile (5.5%). This incidence of positive stools was not significantly different from the 1.3% found in the stools of the other control (outpatient) group, who had a normal bowel habit and no recent exposure to antibiotics.

Although it is suggested that the use of antibiotics facilitates the overgrowth of Clostridium difficile in the stool,18 the effects of sulphapyridine on the faecal flora are less certain. Early studies were contradictory, suggesting both a rise and a fall of anaerobes,19,20 but a recent study reported no change in the anaerobic flora.21 The results of the present study suggest that sulphapyridine encourages the presence of Clostridium difficile in the stools of patients with inflammatory bowel disease, despite its undoubted beneficial effect in the prevention of inflammatory bowel disease relapse.22

Unlike earlier reports,9–11 the present prospective study does not show a relationship between the clinical assessment of inflammatory bowel disease activity and the presence of Clostridium difficile in the stool. Clostridium difficile, however, was significantly more frequent in the stools of those patients with inflammatory bowel disease admitted to hospital. It is remotely possible that these patients may have been infected by a contaminated hospital environment,5,7 and that there is no causal relationship between Clostridium difficile’s presence and disease activity. Alternatively, as the results of Clostridium difficile isolation and the measurement of its cytoxin are not always concordant for the same stool sample,18,23 it is possible that stool analysis only detects a certain degree of faecal
overgrowth with the organism. Indeed, in terms of the pathogenesis of colonic damage, these toxin assays are irrelevant, as the cytotoxin measured in this and most other studies is probably not the toxin that induces colitis. The combination of inexact clinical assessment of disease activity and imprecise detection of either \textit{Clostridium difficile} or its non-pathogenic toxin, may have obscured any correlation between the two events, especially in out-patients with inflammatory bowel disease.

It is also possible that \textit{Clostridium difficile} has no pathogenic role; its proliferation could be facilitated by either a damaged colonic mucosa or by liquid colonic contents during diarrhoea.

Symptomatic improvement of inflammatory bowel disease after uncontrolled vancomycin treatment has been cited as further evidence that \textit{Clostridium difficile} may have a pathogenic role in inflammatory bowel disease. When the results of the present study were analysed at the end of the year, it was clear that two patients (one with ulcerative colitis and the other with Crohn's colitis) had persistently positive stool samples for \textit{Clostridium difficile}. Both patients were ill with continuing colitis despite conventional treatment. They were treated with vancomycin 250 mg qds for 10 days. \textit{Clostridium difficile} and its toxin were eradicated from their stools, but the patients' symptoms did not improve dramatically.

The results of the present study show that \textit{Clostridium difficile} is found with an increased frequency in the stools of patients with inflammatory bowel disease, suggesting that it may be a cause of illness in some of these patients. \textit{Clostridium difficile} was found in the stools of 28% of these patients on one or more occasions during the year. As the laboratory assessment of \textit{Clostridium difficile} or its pathogenic toxin may be imprecise, it is possible that its role in inflammatory bowel disease may even have been underestimated by this study. Eradication of colonic \textit{Clostridium difficile} may induce remission of colitis. This hypothesis could be tested by a placebo-controlled trial of oral vancomycin in patients with acute colitis in whom conventional laboratory assessment for \textit{Clostridium difficile} is negative; a preliminary report of such a study suggests that vancomycin may be of benefit.

\textit{Clostridium} species are important veterinary pathogens, but are controlled by successful inoculation programmes. Similar immunisation in man projects against necrotising enterocolitis. Human immunisation using \textit{Clostridium difficile} toxoid is feasible and would be a practical proposition in a high risk population: patients with inflammatory bowel disease may fulfil this role.

This study was supported by a grant from the Peter Samuel Royal Free Fund.

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*Gut* 1983 24: 713-717
doi: 10.1136/gut.24.8.713

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