

# Spectrum of antibiotic-associated diarrhoea

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**SUMMARY** In an attempt to find the extent to which *Clostridium difficile* could be implicated as the cause of antibiotic-associated diarrhoea, the stools of 53 patients who had diarrhoea after a course of antibiotics were investigated for the presence of *C. difficile* toxin. Ten of the patients (19%) were found to be positive, but the stools of four out of 53 patients without diarrhoea after a course of antibiotics were also found to contain *C. difficile* toxin (7.5%). The titre of toxin in patients both with and without diarrhoea fell within the same range (up to  $10^{-5}$ ). Neither the organism nor its toxin was found in the stool of 26 patients with ulcerative colitis, eight with Crohn's disease, 49 with non-specific diarrhoea, and 27 normal controls. We conclude that, while *C. difficile* is responsible for a proportion of cases of antibiotic-associated diarrhoea, the concentration of toxin is not the sole factor affecting the severity of this disorder.

Pseudomembranous colitis has been reported sporadically for over a century, but it is only since the advent of the antibiotic era that the condition has become relatively common. It had been suggested that the disorder was due to alteration of the colonic flora, but, for many years, no pathogenic organism common to all cases of pseudomembranous colitis could be isolated. However, in recent years it has been shown that *Clostridium difficile* is the causative organism,<sup>1-5</sup> damage being associated with a cytopathic endotoxin which can be neutralised *in vitro* by the antitoxin to *C. sordellii*.

While pseudomembranous colitis is still a relatively rare disorder, diarrhoea occurring after a course of antibiotics is quite a frequent occurrence<sup>6-7</sup> and it was therefore felt that such patients should be investigated to ascertain whether *C. difficile* could also be implicated in these cases.

## Methods

### PATIENTS

Stool samples were collected from 53 patients with diarrhoea during or immediately after a course of antibiotics (Table 1). The patients' symptoms ranged from a mild short-lived episode of diarrhoea of four or more liquid stools per day to severe bloody diarrhoea with systemic illness. The range of anti-

Table 1 Patients studied

	No.	Toxin present in stool	
		(no.)	(%)
Antibiotic-associated diarrhoea	53	10	19
Ulcerative colitis	26	0	
Crohn's disease	8	0	
Non-specific diarrhoea	49	0	
Antibiotics without diarrhoea	53	4	7.5
Normal	27	0	

biotics taken was wide, most having taken a single antibiotic, but some having received combinations of two or three. Stool samples were also studied from 26 patients with active total ulcerative colitis, eight patients with active Crohn's disease with diarrhoea, and 49 patients with non-specific diarrhoea, either with an acute infective episode for which no specific organisms had been found, or with the irritable bowel syndrome. As controls, stools were examined from 53 patients who had received a course of antibiotics for a variety of reasons without developing diarrhoea and 27 patients who had no gastroenterological symptoms and had received no antibiotics for at least two months.

A sample of stool from each patient was cultured specifically for *C. difficile* and faecal filtrates were investigated for the presence of *C. difficile* toxin.

## Techniques

The faecal filtrates were prepared by suspending the stool in normal saline at a dilution of 1 in 2 for

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Table 2 Clinical data of patients with antibiotic-associated diarrhoea

Patient no.	Antibiotic	Reason for administration	Clinical condition	Sigmoidoscopic features	Toxin titre
1	Clindamycin	Sore throat	Severe diarrhoea with systemic illness	Pseudomembrane	10 <sup>-5</sup>
	Clindamycin	Sore throat	Severe blood-stained diarrhoea, systemic illness	Ulceration	10 <sup>-5</sup>
3	Clindamycin	Sepsis of finger	Blood-stained diarrhoea	Loss of vascular pattern	10 <sup>-3</sup>
4	Clindamycin	Varicose ulcers	Severe blood-stained diarrhoea, systemic illness	Ulceration	10 <sup>-4</sup>
5	Amoxycillin	Sore throat	Blood-stained diarrhoea	Loss of vascular pattern	10 <sup>-4</sup>
6	Amoxycillin	Sore throat	Blood-stained diarrhoea	Erythema	10 <sup>-3</sup>
7	Ampicillin	Bronchopneumonia, CVA	Diarrhoea	Not performed	10 <sup>-5</sup>
8	Flucloxacillin	Fractured neck of femur	Severe diarrhoea, systemic illness	Loss of vascular pattern	10 <sup>-5</sup>
9	Cephadrine	Fractured neck of femur	Severe diarrhoea	Loss of vascular pattern	10 <sup>-5</sup>
10	Cotrimoxazole	Chronic bronchitis	Diarrhoea	Not performed	10 <sup>-5</sup>

liquid stools and 1 in 3 or 1 in 4 for the more solid stools. The suspensions were centrifuged at 10 000 g for 30 minutes at 4°C and passed through 0.22 filters. The filtrates were stored at -40°C.

Toxin assay was performed on confluent monolayers of HeLa cells, 0.1 ml of each filtrate being added to 1 ml of culture medium. *C. difficile* toxin was considered to be present when a typical cytopathic effect was seen after 24 or 48 hours and could be neutralised by previous incubation with *C. sordelli* antitoxin. The concentration of toxin was estimated by serial 10-fold dilutions in saline.

Culture of the stool sample was performed under strict anaerobic conditions, initially using modified cycloserine-celoxitin-fructose-egg yolk-agar (CCFA), as described by George *et al.*,<sup>8</sup> but latterly using a medium developed in our own laboratories (Columbia agar (oxid) 40 g/l, egg yolk 5%, Cycloserine 500 µg/ml, cefoxin 16 µg/ml), which we found easier to prepare and use routinely.

## Results

Of the 53 patients who had diarrhoea after a course of antibiotics, there were 10 patients whose stool contained *C. difficile* toxin. In all but one, the organism was also isolated, the exception being an early case occurring before a reliable method of culture had been perfected. One of the 10 patients had histologically proven pseudomembranous colitis, while two had severe colitis without pseudomembrane but with blood and pus in the stool and a systemic illness. Of the remainder, two were not submitted to sigmoidoscopy but the remaining five patients showed evidence of slight inflammation only, with loss of vascular pattern or erythema, although the diarrhoea in three cases was sufficiently severe to be the cause of their admission. The antibiotics administered and the reasons for their administration are shown in Table 2; it can be seen

that, in most cases, the original diagnosis was a fairly trivial problem arising in a previously fit patient.

Neither the organism nor its toxin was isolated from any of the patients with Crohn's disease or ulcerative colitis, even in two whose exacerbation was related to a recent course of antibiotics, and those with non-specific diarrhoea and the normal controls were similarly negative.

However, of those who had received a course of at least seven days' antibiotics without developing diarrhoea, there were four patients whose stool contained *C. difficile* toxin, although the organism was not isolated from two early cases who were seen before we were able to culture *C. difficile* reliably. None of these patients developed diarrhoea during the remainder of their hospital admission (two to three weeks). Antibiotics given and reasons for administration are shown in Table 3.

The concentration of toxin in those patients with diarrhoea ranged from 10<sup>-2</sup> to 10<sup>-5</sup> (Table 2). The concentration in those patients without diarrhoea was either 10<sup>-4</sup> or 10<sup>-5</sup>, in the same range as those with severe colitis (Table 3).

Table 3 Antibiotic treatment in patients with *C. difficile* toxin without diarrhoea

Antibiotic	Condition	Titre
Cephalexin	Hip replacement	10 <sup>-4</sup>
Cephalexin	Hip replacement	10 <sup>-5</sup>
Flucloxacillin	Hip replacement	10 <sup>-5</sup>
Talampicillin	Bronchopneumonia	10 <sup>-4</sup>

## Discussion

*Clostridium difficile* is not commonly found in the adult colon. George *et al.*<sup>9</sup> have estimated its incidence at approximately 2%, although, because of the difficulties of isolating the organism when

present in small quantities, the actual incidence may be higher than this. In children, however, the incidence is greater, being present in up to 50% of neonates<sup>10,11</sup> and 15% of infants between the ages of 1 week and 1 year,<sup>12</sup> possibly as a result of contact with vaginal flora during birth.<sup>13</sup> While no normal adult has yet been shown to have *C. difficile* toxin in the stool, a proportion of healthy neonates have been shown to harbour the toxin without any apparent ill effects.<sup>11,14</sup>

Other workers have studied the incidence of *C. difficile* in antibiotic-associated diarrhoea and other non-antibiotic-related gastrointestinal disorders. Bartlett *et al.*<sup>15</sup> found that nine out of 63 patients with antibiotic-associated colitis without pseudomembrane had *C. difficile* toxin in their faeces, an incidence of 15%, but they failed to isolate either organism or toxin from patients without diarrhoea after a course of antibiotics, although only 14 patients were studied. LaMont and Trnka<sup>16</sup> and Bolton *et al.*<sup>17</sup> have found several cases of inflammatory bowel disease in whom an exacerbation of symptoms has been associated with the identification of *C. difficile* toxin in the faeces. Improvement of the symptoms and eradication of toxin occurred after a course of vancomycin or metronidazole. We were unable to implicate *C. difficile* in exacerbations of inflammatory bowel disease, even in two patients with ulcerative colitis whose exacerbation appeared to be related to a course of antibiotics.

There do not appear to have been any previous reports of patients without diarrhoea after a course of antibiotics but with *C. difficile* toxin in the faeces. Bartlett *et al.*<sup>18</sup> have found that the concentration of toxin in the faecal filtrates has correlated in most cases with the severity of the disease, those for antibiotic associated diarrhoea having a mean of  $10^{-2}$  and those for colitis a mean of  $10^{-3}$  with a maximum of  $10^{-7}$ . However, although in two cases (patients 3 and 6, Table 2) stool samples were not available when symptoms were maximal, the severity of symptoms in the patients studied did not appear to correlate with the concentration of faecal toxin; indeed, in those who failed to develop diarrhoea, the concentration was well within the range found in those patients with severe symptoms. Thus other factors appear to be involved in the pathogenesis of the disorder than the mere presence of a certain concentration of toxin in the colon. Healthy infants seem able to tolerate the presence of *C. difficile* toxin without any ill effects<sup>11,14</sup> and *C. difficile* associated colitis is only rarely reported in children only one of our cases being under the age of 16 years. The reason for this apparent tolerance is not clear; it appears that the infant colon is in some way resistant to the effects of the toxin and it may be

that some adults are able to retain this resistance, or acquire some form of immunity, either local or systemic after exposure in infancy. Bartlett<sup>18</sup> was unable to find any evidence of antitoxin production in the convalescent serum of patients with pseudomembranous colitis, but we have found toxin neutralising activity in the serum of one patient with a one month history of antibiotic-associated diarrhoea;<sup>19</sup> whether such neutralising activity has a protective role has yet to be ascertained.

In conclusion, it would seem that *Clostridium difficile* is responsible for a proportion of cases of antibiotic-associated diarrhoea with a wide spectrum of response, ranging from no symptoms to pseudomembranous colitis, but that the presence of *C. difficile* toxin in the colon is not the sole factor affecting the severity of the disease.

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