

***Clostridium difficile* infection in patients with haematological malignant disease**

Risk factors, faecal toxins and pathogenic strains

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SUMMARY

Two hundred and forty-eight patients from shared oncology and general medical wards were prospectively studied over a 6-month period for carriage of *Clostridium difficile* during an outbreak of clinical disease with an epidemic strain of the organism. Risk factors for infection were assessed. Acute leukaemia and/or its treatment were identified as significantly increasing the risk of infection.

The relationship between the type of *C. difficile* isolated (as defined by a typing system based on the incorporation of [³⁵S]methionine into bacterial proteins followed by gel electrophoresis), the presence of faecal toxins A and B and clinical symptoms were analysed. Carriage of the epidemic strain, type X, had a significant association with symptoms amongst oncology patients, with two thirds of these patients having detectable faecal toxin A and one third detectable faecal toxin B. During an outbreak of *C. difficile*-associated disease, typing the organism and assaying for both faecal toxins in symptomatic patients may be of benefit in determining which patients require specific, urgent treatment.

INTRODUCTION

Whilst several different groups of hospital patients appear to be susceptible to colonization with *Clostridium difficile* (Thompson, Gilligan & Long, 1982), infection with this organism may be of particular clinical significance to immunocompromised patients (Malamou-Lades *et al.* 1983; Morris *et al.* 1984). The aetiology of *C. difficile*-associated disease (CDAD) in an individual patient is multifactorial, involving the interaction of host immune factors in relation to chemotherapy (Fainstein, Bodey & Fekety, 1981), antibiotic use (Tedesco, 1982), and environmental exposure (Kim *et al.* 1981; Bartlett & Taylor, 1984).

A recent report has described a cohort of patients with haematological malignancies infected with the organism and documented the clinical course of CDAD in immunocompromised patients but not in the context of an outbreak (Rampling *et al.* 1985). During an epidemiological study of *C. difficile* at St Bartholomew's Hospital using a new typing scheme for the organism (Tabaqchali

et al. 1984), it became apparent that a serious clinical outbreak of the disease, which was associated with several deaths, was occurring on the oncology wards. This provided the opportunity to initiate a prospective epidemiological study for a period of 6 months to determine the carriage and acquisition of *C. difficile* amongst immunocompromised patients and medical patients who shared the same wards (associated medical patients). The results provide evidence for nosocomial acquisition and cross-infection amongst patients with an epidemic strain of the organism (Heard *et al.* 1986).

In this paper we report details of the risk factors for acquisition of the organism during this outbreak and describe the possible relationships between the type of *C. difficile* isolated in the presence of faecal toxins and the symptoms associated with it.

PATIENTS AND METHODS

Study population

Two hundred and forty-eight patients from male and female oncology/general medical wards, were prospectively screened over a 6-month period for the presence of *C. difficile* in faecal specimens. There were approximately equal numbers of oncology patients and medical patient sharing each ward, separated by a partition down the centre of the ward. Nursing staff and bath and toilet facilities were shared by all patients. Specimens (either samples or rectal swabs, if necessary) were taken within 48 h of admission to the ward and then weekly thereafter for the duration of the patient's stay in hospital. Sigmoidoscopy was not performed since most patients were thrombocytopenic either in association with the treatment of their malignancy or due to their disease. Further details have been reported elsewhere (Heard *et al.* 1986).

Case notes from the patients participating in the screen were analysed and information concerning the underlying illness, its treatment, especially with regard to antibiotics (both intravenous and those administered orally for gastrointestinal decontamination), chemotherapy, the number of recurrent episodes of *C. difficile*-associated disease and the periods and extent of neutropenia were collected. Symptoms and signs were recorded by clinical staff and analysed in relation to the presence of faecal isolates of the organism, the type of strains obtained and the amount of toxins A and B detected in faecal samples.

Isolation of C. difficile

Faecal specimens were cultured for 48 h anaerobically on selective media for *C. difficile* (cycloserine, cefoxitin and fructose agar [CCFA], Oxoid) (George *et al.* 1979). The organism was identified presumptively by its macroscopic and Gram stain morphology, its smell and its ability to fluoresce under long-wave ultraviolet light. The characteristic pattern of the volatile fatty acids produced on gas liquid chromatography was used to confirm the identity of the organism (Holdeman, Cato & Moore, 1977).

Toxin A and B assay

Faecal samples from which *C. difficile* had been cultured or from patients known either to have had the organism on previous culture or to have had a clinical

syndrome suggestive of the diagnosis were stored in glycerol at -70°C and were subsequently analysed for toxins A and B. Toxin B was assayed using a Hep 2 cell line by standard methods (George *et al.* 1978).

A direct sandwich ELISA technique was used for the quantitative detection of toxin A as described by Redmond *et al.* (1985). After defrosting the faecal samples described above to room temperature, aliquots were centrifuged for 10 min (12000 g; Eppendorf centrifuge) and 50 μl of supernatant was applied directly to microtitre wells (Nunc) coated with antitoxin A. The assay was quantified spectrophotometrically at 492 nm (Biorad ELISA plate reader) by comparison of the quantities of toxin A present in the test solutions with standard samples of toxin A. Appropriate controls were included in each assay. Purified toxin A derived from dialysates of *C. difficile* by flat-bed electrophoresis and ion-exchange chromatography and rabbit IgG antitoxin A conjugated to horseradish peroxidase were gifts from Dr J. Stephens (University of Birmingham).

Typing of strains

The typing scheme used in this survey was developed at this hospital and has been reported in detail elsewhere (Tabaqchali *et al.* 1984, 1986).

Statistical methods

Chi-square analysis was used to determine the significance of risk factors in relation to the type of organism isolated and clinical symptoms.

RESULTS

Carriage rate of C. difficile

As previously reported 49 of the 135 oncology patients screened had an isolate of *C. difficile*, whilst 13 of the 113 associated medical patients carried the organism (Heard *et al.* 1986). There was an almost equal distribution amongst male (26) and female (23) oncology patients, with an age range of 18–78 years (mean 49 years) and a normal distribution over that range. Three of the 13 associated medical patients were female and 10 were male. The age range was 50–89 years, with a mean age of 67 years.

Analysis of risk factors

The possible risk factors associated with infection by the organism were considered. Sixty-seven of the 86 oncology patients (78%) who failed to acquire the organism had received antibiotics (Table 1), whilst 80% (39/49) of the infected patients had also been treated with them ($P > 0.2$). Of the 100 medical patients screened who did not become infected, 25 had received antibiotics whilst 5 of the 13 medical patients who acquired the organism had also received antibacterial agents. The administration of antibiotics, combinations of antibiotics (tobramycin and cephalosporin, tobramycin and piperacillin, erythromycin and chloramphenicol), steroids and chemotherapy, and the relevance to neutropenia were all studied but no differences could be shown between the infected and non-infected groups. Only acute leukaemia or its treatment (using a protocol involving the administration of cytosine arabinoside, adriamycin and 6-thioguanine)

Table 1. *Clinical details of patients screened for C. difficile*

	Oncology patients (135)		Medical patients (113)	
	Culture +ve 49 (%)	Culture -ve 86 (%)	Culture +ve 13 (%)	Culture -ve 100 (%)
No. of patients...				
Males	26 (53%)	50 (58%)	10 (77%)	50 (50%)
Females	23 (47%)	36 (42%)	3 (13%)	50 (50%)
Mean age (years)	49	55.1	67	62.9
Diagnosis				
Acute leukaemia	37 (76%)	9 (10%)	0	0
Other oncological disease	12 (24%)	77 (90%)	3 (23%)	0
Other medical conditions	0	0	10 (77%)	100
Cytotoxics given	41 (83%)	60 (70%)	0	0
Antibiotics given	39 (80%)	67 (78%)	5 (38%)	25 (25%)
Acute GI symptoms				
Abdominal pain	18 (37%)	6 (7%)	2 (15%)	2 (2%)
Diarrhoea	33 (67%)	15 (17%)	4 (31%)	2 (2%)

Table 2. *Relationship between acute leukaemia and/or its treatment and isolation of Clostridium difficile*

	Positive isolate	Negative isolate
Acute leukaemia	37	9
Other oncological disease	12	77

$$\chi^2 = 58.7; P < 0.001.$$

appeared to constitute a significant risk factor for acquisition of the organism ($P < 0.001$, Table 2). Thirty-seven of the 49 culture-positive patients had acute leukaemia. Only nine patients followed through the screening period with acute leukaemia had negative cultures for *C. difficile*.

Typing of C. difficile

The typing results based on radiolabelled SDS-PAGE of the isolates obtained are shown in Table 3. Seventy-one per cent (35/49) of oncology patients with *C. difficile* carried the epidemic strains, type X, whilst the rest carried a variety of other strains. Three medical patients sharing the same ward environment carried the epidemic strain.

Frequency of isolation of C. difficile

Thirty-two of the 49 oncology patients had the organism isolated from more than one stool specimen (range of isolates 2-10). Twenty-two patients had multiple episodes of infection (median 2). An episode was defined as the isolation of the organism in relation to a new cycle of chemotherapy or new course of antibiotics. Fifteen patients had two episodes of infection, 2 had three episodes, 3 had four, one had five episodes and the last had six different episodes of infection

Table 3. *Types of Clostridium difficile isolated*

Types of <i>C. difficile</i> ...	A	B	C	D	E	W	X	Y	Z
Oncology patients	1	0	0	1	5	1	35	6	0
Associated medical patients*	1	3	0	1	3	0	3	1	0

* One isolate died before typing could be performed.

Table 4. *Types of Clostridium difficile isolated from symptomatic oncology patients*

	No. of patients	Epidemic type (X)	Non-epidemic type
Symptomatic patients	33	29	4
Asymptomatic patients	16	6*	10

$\chi^2 = 14.72$; $P < 0.001$.

* 3 patients, early treatment; 1 patient, no antibiotics, chemotherapy or neutropenia; 1 patient, non-Hodgkins lymphoma - no clinical symptoms; 1 patient, acute leukaemia - no clinical symptoms.

with the organism. Seven of these 22 patients failed to clear the organism between episodes, whilst in the remaining 15 patients the organism was not cultured between treatment courses. The strains isolated from multiple faecal samples examined during one episode and those isolated during recurrent episodes were the same in all but three patients. Follow-up faecal samples from 11 patients were not available so that the presence of persistent carriage could not be analysed in these patients. Sixteen patients had one episode of CDAD. Fourteen of these patients cleared the organism from the stool (three did not receive specific treatment with vancomycin), one failed to clear the organism despite treatment and one other patient died before treatment could be instituted.

Relationship between the type of C. difficile and symptoms

Table 4 demonstrates the relationship between the type of *C. difficile* isolated and symptoms amongst the oncology patients. Thirty-three symptomatic patients (67%) had diarrhoea, as defined by complaints of loose stool more than twice in 24 h. In addition, 18 patients experienced some abdominal pain and 26 had a pyrexia in association with diarrhoea (Table 1). Twenty-nine of these patients (88%) were infected with type X, whilst the remaining four symptomatic patients carried non-epidemic strains. Ten of the 16 asymptomatic oncology patients carried non-epidemic strains, while six were colonized with the epidemic strain. Four of the colonized medical patients developed diarrhoea, whilst only 15 oncology patients (17%) developed diarrhoea in the absence of an isolate of *C. difficile*.

Faecal toxins

Three hundred and four faecal samples were examined for toxin B and 260 for toxin A. Thirty of the 304 stools assayed (involving 16 different patients) had detectable toxin B. Eleven of 33 symptomatic oncology patients had toxin B

Table 5. *Toxins A and B in faecal samples obtained from oncology patients*

	Toxin A detected	Toxin A not detected	Toxin B detected	Toxin B not detected
Symptomatic patients				
Type X*	19	8	11	17
Non-X type	3	1	2	3
Asymptomatic patients				
Type X	6	0	2	4
Non-X type	10	0	1	9

* Two strains not tested for toxin A.

detectable in their faecal samples and two of these were non-X strains (C and E) whilst amongst asymptomatic patients with detectable faecal toxin B, two carried the epidemic strain and one carried strain E (Table 5).

Toxin A was far more commonly found in faecal samples than was toxin B. One hundred and eighteen faecal samples from 54 patients had detectable levels of toxin A (4 patients were not tested and 4 patients had no toxin A detected). Amongst the faecal samples positive for the toxin, 64 were culture positive for *C. difficile* and 54 were negative on culture for the organism. All of the patients with culture negative samples positive for toxin A had had the organism detected in previous faecal samples, although on one occasion stools from a symptomatic patient were positive on toxin A assay 2 weeks prior to the organism being cultured from the faeces. Sixty-seven of the remaining 142 faecal specimens negative for this toxin were positive on culture for the organism and 75 were negative both on culture and for the toxin. Eleven culture positive patients had no detectable faecal toxin A.

DISCUSSION

Previous studies have reported the epidemiology of CDAD in cohorts of patients with haematological malignant disease (Morris *et al.* 1984; Rampling *et al.* 1985; Heard *et al.* 1986) but could identify no risk for colonization with *C. difficile*. In this study the isolation of *C. difficile* from patients with acute leukaemia occurred more commonly than expected, even during an outbreak of the disease. Forty-six of the 138 oncology patients screened had acute leukaemia and only 9 were negative during the screening period ($P < 0.001$). Acute leukaemia is therefore an important risk factor, but whether it is the disease itself or its treatment with agents which are toxic to the gastrointestinal mucosa (Slavin, Dias & Soral, 1978), is not known.

The relevance of neutropenia and of specific antibiotic or chemotherapeutic regimes could not be assessed. All patients received antibiotics, chemotherapy or both at some time during their treatment, and were either neutropenic or had a malignant abnormality of their haematopoietic system by the time they were investigated. Length of stay in hospital was not analysed since the oncological patients involved in this study, particularly the patients with acute leukaemia, had frequent and multiple admissions to hospital with recurrent exposure to the outbreak strain.

Rogers *et al.* (1981) have noted the association between prophylactic non-absorbable antibiotic regimes and colonization of the gastrointestinal tract in children receiving bone marrow transplantation. In this present study, only a few of the 46 patients with acute leukaemia tolerated the non-absorbable antibiotics (framycetin, nystatin, colistin) which were prescribed for the entire treatment period (6 months). The sudden cessation of non-absorbable antibiotics for short periods of time (usually only a few days), especially when patients were neutropenic and unwell, may encourage colonization of the gastrointestinal tract with *C. difficile*. Two of the nine patients with acute leukaemia who did not become infected failed to comply with taking the non-absorbable antibiotics over an extended period of time (several weeks), whilst the other seven patients either took none at all (five patients) or took them continuously (two patients). One patient who was screened over 6 months during his treatment for acute leukaemia consistently took the non-absorbable antibiotic regime as prescribed. He failed to acquire the organism until his final course of chemotherapy, when he was unable to comply with the non-absorbable antibiotic regime for 3 days during a febrile, neutropenic episode. He subsequently became infected with the organism and developed symptoms consistent with CDAD. Thus the intermittent use of non-absorbable agents may encourage the rebound colonization of the gastrointestinal tract with *C. difficile* and make it more susceptible to infection with the organism.

Fourteen oncology patients were found to be carrying strains other than the epidemic strain, four of whom had symptoms of CDAD. By contrast, 88% (29/33) of oncology patients who were symptomatic typed as X (Table 4) whilst only 38% (6/16) patients who were asymptomatic carried this type. This was highly significant at the 0.1% level and suggests that the epidemic strain, in the presence of precipitating factors, may have been particularly virulent. Typing of clinical isolates may therefore indicate which patients require early treatment and special management and may be an important tool in distinguishing between colonization and infection with the organism.

The presence of a virulent type of *C. difficile* (i.e. X strain) in association with precipitating factors such as acute leukaemia or its treatment involving the use of chemotherapeutic agents and antibiotics, is suggestive that the diarrhoea experienced by these patients can be attributed to *C. difficile* infection and may not be due solely to the effects of chemotherapy. Only 17% of the oncology patients screened who did not acquire *C. difficile* had diarrhoea, despite receiving comparable exposure to cytotoxics and antibiotics. This sheds some doubt on the frequent clinical claim that diarrhoea in such patients is an unfortunate but nonetheless anticipated side-effect of the treatment itself. It may be that a large number of patients are suffering from infective diarrhoea caused by *C. difficile* for which specific treatment is available. In a 1-year follow-up of patients on the oncology wards following this prospective survey, 50% of symptomatic patients with diarrhoea were infected with *C. difficile* (unpublished observation).

Toxin testing performed on faecal specimens suggests that when toxin is detected in samples from symptomatic patients these symptoms may be attributable to *C. difficile* infection. Faecal toxin B assay is helpful in making a diagnosis of CDAD when it is positive, and the titre of toxin may well be indicative

of the presence of a pseudomembrane. Nonetheless, toxin B does not appear to be a *sine qua non* of even pseudomembranous colitis (PMC) since Burdon *et al.* (1981) found that 7 of 33 patients (21 %) with rectal pseudomembranes and *C. difficile* in the stool failed to have detectable faecal toxin B. In an epidemic situation such as described here, toxin B assay may be of benefit in helping to distinguish between colonization and infection, since 13 of 16 faecal specimens from oncology patients which were positive for this toxin were associated with the epidemic strain. However, since overall, only one-fifth of culture positive patients in this survey were also positive for toxin B, its usefulness appears limited, for whilst a positive result can confirm a clinical impression of infection, a negative one cannot exclude the diagnosis.

Two other factors may have contributed to the low incidence of faecal toxin B detection in this study. First, faecal specimens were screened at a starting dilution of 1:100. Other studies have used starting titres of 1:10 or lower and hence have assayed only for the presence or absence of the toxin (Viscidi, Willey & Bartlett, 1981). A wider dilution range was used in this study in order to consider the relevance of the toxin titre to both the type of organism and the symptoms produced. More faecal samples might well have been positive had a lower dilution been used as a starting point. In addition, treatment with vancomycin (Keighley *et al.* 1978) was commenced early during this outbreak because of the severity of the symptoms and the high clinical presumption that *C. difficile* was accounting for them. Many of the 304 faecal samples assayed for toxin B were specimens from patients who were receiving vancomycin. Whilst only 10% of all faecal samples examined were positive for toxin B, nearly 40% of symptomatic patients (13/33) had detectable levels of this toxin.

Toxin A was found with greater frequency in faecal samples than toxin B and may be a more sensitive indicator of infection with the organism. Stephens *et al.* (1985) screened 300 faecal samples from patients with diarrhoea and found that those which were negative on culture for the organism were also negative for toxin A. In this study 54 of the 118 toxin A positive faecal samples (45.7%) were culture negative for the organism. Screening faecal samples for toxin A rather than for toxin B may therefore be an effective means of identifying infected patients.

This is the first large, prospective cohort study of oncology patients with CDAD which considers the type of organism isolated in relation to the presence of faecal toxins and the risk factors associated with its acquisition. It demonstrates that acute leukaemia or its treatment appears to constitute an important risk factor both for acquisition of the organism and for symptomatic disease within an outbreak situation. Screening for the organism and/or its toxins may therefore be important on units that cannot maintain strict enteric precautions between patients in order to help prevent cross-infection. Whilst the importance of a particular antibiotic regime in the context of such an outbreak is difficult to assess, intermittent use of bowel decontaminants may be relevant in making the immunocompromised bowel more susceptible to infection with the organism. Both the type of *C. difficile* isolated and the detection of its toxins in faecal samples, especially toxin A, may help in deciding when an isolate is clinically relevant (Tabaqchali *et al.* 1984; Delmee, Homel & Wauters, 1985). In view of increasing

animal evidence that non-pathogenic strains may help to protect the bowel from pathogenic strains (Wilson & Sheagren, 1983; Barclay & Borriello, 1981), typing the organism may be of benefit in determining those patients who require early, specific treatment for CDAD.

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REFERENCES

- BARCLAY, F. & BORRIELLO, S. P. (1981). *Clostridium difficile* and colonization resistance. In *Antibiotic-associated-diarrhoea and Colitis* (ed. S. P. Borriello), pp. 80–87. The Hague: Martinus Nijhoff.
- BARTLETT, J. G. & TAYLOR, N. S. (1984). Antibiotic associated colitis. In *Medical Microbiology*, vol. 1 (ed. C. S. F. Easmon, J. Jelkaszewicz, M. R. W. Brown and P. A. Lambert), pp. 1–48. London: Academic Press.
- BURDON, D. W., GEORGE, R. H., MOGG, G. A. G., ARABI, Y., JOHNSON, M., ALEXANDER-WILLIAMS, J. & KEIGHLEY, M. R. B. (1981). Faecal toxin and severity of antibiotic-associated pseudomembranous colitis. *Journal of Clinical Pathology* **32**, 548–551.
- DELMEE, M., HOMEL, M. & WAUTERS, G. (1985). Serogrouping of *Clostridium difficile* strains by slide agglutination. *Journal of Clinical Microbiology* **21**, 323–327.
- FAINSTEIN, V., BODEY, G. P. & FEKETY, R. (1981). Relapsing pseudomembranous colitis associated with cancer chemotherapy. *Journal of Infectious Diseases* **143**, 865.
- GEORGE, R. H., SYMOND, J. M., DINNOCK, F., BROWN, J. D., ARABI, Y., SHINAGAWA, N., KEIGHLEY, M., ALEXANDER-WILLIAMS, J. & BURDON, J. D. W. (1978). Identification of *Clostridium difficile* as a cause of pseudomembranous colitis. *British Medical Journal* **i**, 695.
- GEORGE, W. L., SUTTER, V. L., CITRON, D. & FINEGOLD, S. M. (1979). Selective and differential medium for isolation of *Clostridium difficile*. *Journal of Clinical Microbiology* **9**, 214–219.
- HEARD, S. R., O'FARRELL, S., HOLLAND, D., CROOK, S., BARNETT, M. J. & TABAQCHALI, S. (1986). The epidemiology of *Clostridium difficile* with use of a typing scheme: nosocomial acquisition and cross-infection among immunocompromised patients. *Journal of Infectious Diseases* **1**, 159–162.
- HOLEMAN, L. V., CATO, E. P. & MOORE, W. E. C. (eds) (1977). *Anaerobe Laboratory Manual*, 4th edn, p. 98. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.
- KEIGHLEY, B. R. B., BURDON, D. W., ARABI, Y., ALEXANDER-WILLIAMS, J., THOMPSON, H., YOUNGS, D., JOHNSON, M., BENTLEY, S. L., GEORGE, R. H. & MOGG, G. A. G. (1978). Randomised controlled trial of vancomycin for pseudomembranous colitis and postoperative diarrhoea. *British Medical Journal* **ii**, 1667–1669.
- KIM, K. H., FEKETY, R., BATIS, D. H., BROWN, D., CUDMORE, M., SILVA, J. & WATER, D. (1981). Isolation of *Clostridium difficile* from the environment and contacts of patients with antibiotic-associated colitis. *Journal of Infectious Diseases* **1**, 42–50.
- MALAMOU-LADAS, H., O'FARRELL, S., NASH, J. Q. & TABAQCHALI, S. (1983). Isolation of *Clostridium difficile* from patients and the environment of hospital wards. *Journal of Clinical Pathology* **36**, 88–92.
- MORRIS, J. G., JARVIS, W. R., NUVEY-MONTIEL, O. L., TOWNS, M. L., THOMPSON, P. S., DOWELL, V. R., HILL, E. O., VOGLER, W. R., WINTON, E. F. & HUGHES, J. M. (1984). *Clostridium difficile*: colonization and toxin production in a cohort of patients with malignant haematological disorders. *Archives of Internal Medicine* **144**, 967–969.
- RAMPLING, A., WARREN, R. E., BEVAN, C. E., HOGGARTH, C. E., SWIRSKY, D. & HAYHOE, F. G. J. (1985). *Clostridium difficile* in haematological malignancy. *Journal of Clinical Pathology* **38**, 445–451.

- REDMOND, S. C., KETLEY, J. M., MITCHELL, T. J., STEPHENS, J., BURDON, D. W. & CANDY, D. C. A. (1985). Detection of *Clostridium difficile* enterotoxin (toxin A) by ELISA and other techniques. In *Isolation and Identification of Microorganisms of Medical and Veterinary Importance* (ed. C. H. Collins and J. M. Grange), Society for Applied Bacteriology Technical Series 21, pp. 237-249. London: Academic Press.
- ROGERS, T. R., PETROU, M., LUCAS, C., CHUNG, J. T. N. & BARRETT, A. J. (1981). Spread of *Clostridium difficile* among patients receiving non-absorbable antibiotics for gut decontamination. *British Medical Journal* **283**, 408-409.
- SLAVIN, R. E., DIAS, M. B. & SORAL, R. (1978). Cytosine arabinoside induced gastrointestinal toxic alterations in sequential chemotherapeutic protocols. A clinicopathologic study of 33 patients. *Cancer* **42**, 1747-1759.
- STEPHENS, J., WILKINS, T., KRIVAN, H., STILES, B., CARMEN, R. & LYERLY, D. (1985). Clostridial toxins active locally in the gastrointestinal tract. In *Microbial Toxins and Diarrhoeal Disease* (Ciba Foundation Symposium), pp. 230-241. London: Pitman.
- TABAQCHALI, S., O'FARRELL, S., HOLLAND, D. & SILMAN, R. (1984). Typing scheme for *Clostridium difficile*: its application in clinical and epidemiological studies. *Lancet* *i*, 935-938.
- TABAQCHALI, S., O'FARRELL, S., HOLLAND, D. & SILMAN, R. (1986). Method for typing of *Clostridium difficile* based on PAGE of ³⁵S-Methionine-labelled proteins. *Journal of Clinical Microbiology* **23**, 197-198.
- TEDESCO, F. J. (1982). Pseudomembranous colitis: pathogenesis and therapy. *Medical Clinics of North America* **3**, 655-664.
- THOMPSON, G. M., GILLIGAN, P. H. & LONG, S. S. (1982). Interrelationships of stool flora and *Clostridium difficile* in children with cystic fibrosis. *Proceedings of the 22nd International Conference on Antimicrobial Agents and Chemotherapy*. Abstract 594.
- VISCIDI, R., WILLEY, S. & BARTLETT, J. G. (1981). Isolation rates and toxigenic potential of *Clostridium difficile* isolates from various patient populations. *Gastroenterology* **81**, 5-9.
- WILSON, K. H. & SHEAGREN, J. N. (1983). Antagonism of toxigenic *Clostridium difficile* by non-toxigenic *C. difficile*. *Journal of Infectious Diseases* **4**, 733-735.