CLOSTRIDIUM DIFFICILE ASSOCIATED DIARRHOEA: A REPORT OF SEVEN CASES

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SUMMARY

Seven cases of *C. difficile*-associated diarrhoea were studied. *C. difficile* was isolated and or its cytotoxin was detected in the faeces of these patients. Clinically two distinct forms (syndrome) were seen: the severe PMC with a high mortality rate and the diarrhoeal form with no associated mortality. Predisposing factors included serious underlying disease, preceeding antimicrobial therapy and major operative procedures.

Greater awareness of this condition and good prospective clinical and laboratory based studies are necessary for the further understanding of *C. difficile* associated illness.

SING MED J. 1988; 29: 504 — 507

INTRODUCTION

The anaerobe *Clostridium difficile* and its exotoxin have been established as causes of pseudomembranous colitis (PMC) (1,2), but the organism is being increasingly recognized to cause a spectrum of diseases associated with the gastrointestinal tract. These include antiobiotic-associated diarrhoea and nonspecific colitis as well as diseases not related to prior antimicrobial therapy such as diarrhoea of apparently spontaneous onset(3,4) exacerbations of inflammatory bowel disease(5) and diarrhoea related to antineoplastic treatment(6).

We report 7 patients seen at the University Hospital, Kuala Lumpur, over the past 3 years and who presented with profuse diarrhoea associated with *C. difficile* in order to highlight the role of this organism and the predisposing risk factors related to this syndrome.

BACTERIOLOGICAL METHODS

Stool from patients with a provisional diagnosis of PMC was cultured for enteric pathogens by standard methods and processed for *C. difficile* as follows: fresh stool was inoculated onto plates containing *C. difficile* agar with 7% defibrinated horse blood, D-cycloserine and cefoxitin supplements (Oxoid). The stool was also processed in a similar manner after "bench alcohol shock" treatment(7). The cultures were incubated at 37°C with a gaspack anaerobic system (Oxide); after 48 hours, *C. difficile* colonies were provisionally identified by their colonial morphology, Gram staining reaction and chartreuse-green flourescence under long wavelength ultraviolet light. Isolates were later confirmed by fatty acid profiles using gas-liquid chromatography.

In addition, the stool were examined for the presence of *C. difficile* cytotoxin by tissue culture assay systems using Vero cells. Samples showing cytotoxic effect were titrated to determine the titre of cytotoxin activity. Specificity of cytotoxin activity was determined by neutralisation tests using *Clostridium sordelli* antiserum (Wellcome Research Laboratories).

CASE REPORTS

The case notes of the seven patients with *C. difficile*-associated diarrhoea were reviewed. Table 1 summarises the relevant clinical and laboratory findings.

All the patients had profuse and watery diarrhoea with or without mucus but none had bloody diarrhoea.

There were 4 females and 3 males and their ages ranged from 29 to 70 years. Only 2 were over 60 years of age. Serious underlying diseases were present in all the cases and 3 patients had recently undergone major operative procedures.

A history of preceeding antimicrobial therapy was present in only 5 patients but there was no predominance of any paticular antimicrobial agent. Although underlying malignanacies were present in 2 patients, only one had been on cytotoxic chemotherapy albeit for a few days but more significantly, she had been on multiple antibiotic therapy previously (case 1).

The seven cases fell into two groups — firstly those who were seriously ill due to both profuse diarrhoea as well as underlying disease (cases 1-4) and secondly, those who were not so ill (cases 5-7). Endoscopies were performed on 2 of the ill patients and pseudomembranous plagues were visualized in both. *C. difficile* cytotoxin was detected in all

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Table 1. Summary of clinical and laboratory data.

Case	Sex/ Age (yrs)	Underlying Disease/Procedure	Antimicrobials Used	C. difficile Isolation	C. difficile Cytotoxin (titre)	Proctoscopy/ Sigmoidoscopy	Specific Treatment	Outcome
1.	F/29	1. carcinoma of cervix 2. post Wertheim's hysterectomy	cotrimoxazole gentamicin metronidazole nitrofurantoin nalidixic acid ampicillin	positive	positive (10^6)	not done	nil	— died of PMC
2.	M/63	1. hypertension 2. diabetes meilitus 3. cerebrovascular accident 4. aspiration pneumonia 5. recurrent urinary tract infection	penicillin cloxacillin gentamicin pipemidic acid nalidixic acid	positive	positive (10^2)	not done	nil	— diarrhoea persisted — died of acute myocardial infarction
3.	M/48	1. diabetes mellitus 2. hepatic failure 3. renal failure 4. cerebrovascular accident 5. urinary tract infection	cefoperazone	positive	positive (10^0)+	PP* seen	nil	— died of PMC
4.	F/78	1. carcinoma of the ovary 2. ischaemic. heart disease 3. post-laparotomy	nil	negative	positive (10^0) +	PP* seen	metroni-dazole	— diarrhoe persisted — died of PMC
5.	F/38	1. chronic rheumatic heart disease 2. post-mitral valve replacement	cloxacillin	positive	positive (10^0) +	normal	nil	— diarrhoea stopped — dis-charged well
6.	M/54	1. hypertension 2. gout 3. bleeding haemorrnoids 4. acute-on-chronic renal failure 5. peritoneal dialysis	cloxacillin cefoperzone ceftazidime gentamicin cotrimoxazole	negative	positive (10^0) +	normal	nil	— diarrhoea stopped — dis-charged well
7.	F/34	1. systemic lupus erythematosus	nil	positive	negative	normal	metroni-dazole	— diarrhoea stopped — dis-charged well

* PP = pseudomembranous plaque. + 10^0 = undiluted sample.

4 (titres of 10^0-10^6) and *C. difficile* was isolated in 3 patients. One patient(case 4) was treated with metronidazole but her diarrhoea persisted. Three patients died of PMC (cases 1,3, and 4) while another died of acute myocardial infarction.

Endoscopies were essentially normal in the 3 not so ill patients. Cytotoxin was detected and *C. difficile* was cultured from the stool of one of them while the remaining 2 patients were either culture or toxin positive. As in the first group, metronidazole given to one patient (case 7) was not successful in the alleviation of her diarrhoea, but she improved subsequently with symptomatic treatment. The remaing two patients were treated symptomatically and their diarrhoea stopped. All three patients were discharged well.

The case histories of 2 patients are presented in greater detail.

Case 1.

A 29 year old Malay female had a Wertheim's radical hysterectomy performed for stage 1B adenocarcinoma of the cervix, under cotrimoxazole and gentamicin prophylaxis. As subsequent histopathological evaluation revealed metastases to the pelvic lymph nodes with vascular and lymphatic permeation by tumour cells in the cervix, she was started on adjuvant cytotoxic chemotherapy (cisplatinum, vinblastine and bleomycin) on the 8th post-operative day. On the 14th post-operative day she developed severe *Escherichia coli* urinary tract infection and was treated with nitrofurantoin, followed by nalidixic acid and ampicillin. She subsequently developed severe diarrhoea and septicaemia whilst on the above antibiotics and died 5 days later. *C. difficile* was isolated from the stool and cytotoxin up to a titre of 10^6 was also detected.

Case 3.

A 48 year old male with diabetes mellitus, hepatic and renal failure was admitted with a recent history of cerebrovascular accident and septicaemia secondary to urinary tract infection. Blood and urine cultures grew Klebsiella sp. and he was treated with cefoperazone for 2 weeks. He had a stormy stay in the hospital and 40 days after admission developed severe diarrhoea. Proctoscopy revealed pseudomembranous plaques. The patient was managed conservatively for this but his general condition deteriorated and 6 days after onset of diarrhoea, he returned home where he died. *C. difficile* was isolated from the stools and the cytotoxin assay was positive (titre 10^9).

DISCUSSION:

The role of *C. difficile* in antibiotic-associated diarrhoea and PMC has been well established (1,2). A diagnosis of PMC by histology alone of biopsied materials has been reported in Malaysia(8) while in Singapore, diagnosis was made by a combination of histology and detection of *C. difficile* cytotoxin(9). But we are not aware of any local reports on the isolation of *C. difficile* from patients with antibiotic-associated diarrhoea and PMC.

The reported spectrum of diarrhoeal diseases associated with *C. difficile* was seen in our seven cases. Two distinct "syndromes" were evident — the severe and life-threatening PMC with an observed mortality of 75% and another of profuse diarrhoea with no macroscopic evidence of colitis and not associated with any mortality.

Bartlett(10) reported that in adults with endoscopic evidence of colitis, 95% of them had *C. difficile* cytotoxin and 90% had *C. difficile* organisms in their stool. But in our small series this high degree of correlation between colitis and the presence of cytotoxin and/or *C. difficile* in stools was not very evident. However, it must be recognised that about 50% of neonates (11) and 3% of healthy adults (12) are said to be asymptomatic carriers of this organism in their gut.

Recognised risk factors in the development of *C. difficile* related colitis are, preceeding antimicrobial or antineoplastic therapy, advanced age and the female status. Severe underlying disease, polyantimicrobial therapy and major operative procedures have been the main predisposing factors in our series.

Specific antimicrobial therapy may not be necessarv in all patients with *C. difficile* associated syndromes. Treatment with vancomycin or metronidazole may be indicated in patients not responding to supportive therapy in the high risk group as well as those who are severely ill and toxic. Two of our patients treated with metronidazole 400 mg tds did not show dramatic responses. Perhaps, higher doses of metronidazole, 500 mg to 1000 mg tds for 7 days(13) may have been more effective as the drug, being well absorbed from the gastrointestinal tract, may not be present in adequate concentrations with low doses. Vancomycin, however, remains the drug of choice in severe infections.

Local epidemiological data is lacking regarding the importance of this organism in antibiotic-associated diarrhoea, PMC and other gastrointestinal diseases. Good prospective clinical as well as laboratory studies (histological and bacteriological) are essential to shed further light on the *C. difficile* dilemma.

ACKNOWLEDGMENTS

The authors would like to thank Mrs Lingga Teo for performing the cytotoxin assays and Dr Hamimah Haji Hassan for the GLC analysis.

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