Evidence for cross-infection in an outbreak of *Clostridium difficile*-associated diarrhoea in a surgical unit

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Summary. Environmental studies were performed in a hospital outbreak of *Clostridium difficile*-associated diarrhoea. Transmission was associated with the sluice room and the storage room where medical equipment was found to be contaminated with *C. difficile*. Typing of isolates by antibiotic-susceptibility patterns and profiles of EDTA-extracted proteins showed the presence of an "epidemic" strain common to the majority of patients and environmental sites. Control of the outbreak was achieved by improvement of environmental hygiene and use of disposable equipment.

Introduction

The epidemiology of *Clostridium difficile*-associated diarrhoea has not yet been fully clarified. Several studies have reported that the environment may be a source of infection (Mulligan *et al.*, 1980; Fekety *et al.*, 1981; Kim *et al.*, 1983; Hall *et al.*, 1985; Cumming *et al.*, 1986). More information about the epidemiology of *C. difficile* infection can be acquired only if a typing system is used.

We studied the circulation of *C. difficile* in a surgical unit where, in a six week period, 10 patients experienced *C. difficile*-associated diarrhoea. Stools from hospital staff and environmental samples were cultured for *C. difficile* and typing procedures were performed on the isolates.

Materials and methods

Patients

Patients were deemed to be affected by *C. difficile*-associated diarrhoea if they had three or more bowel movements with liquid faeces per day and had received antimicrobials, and if *C. difficile* was isolated from cultures of their faeces. The presence of faecal *C. difficile* cytotoxin was determined by conventional techniques (Chang *et al.*, 1979).

Environmental studies

Environmental cultures (150 samples) were obtained from patient-related zones, the nurses room, the sluice

room and adjoining storage room and equipment therein. Samples were obtained by swabbing an area roughly 10 cm in diameter with sterile cotton-tipped swabs, previously moistened with sterile saline (0.85% NaCl). A CCFA plate (George *et al.*, 1979) was then inoculated with the swab. Plates were incubated at 37°C in an anaerobic chamber for 48–72 h before examination and for up to 5 days before being discarded.

Samples were also obtained from the fingers of the only nurse who was attending patients during the environmental study. Stool samples were obtained from all 35 staff members and nine patients who did not have diarrhoea for culture for *C. difficile*.

Typing of isolates

Isolates were typed on the basis of (1) antibiotic susceptibility patterns by the agar dilution method (Sutter *et al.*, 1980) with chloramphenicol, clindamycin, tetracycline, erythromycin and rifampicin, and (2) profiles of EDTA-extracted surface proteins obtained by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The method of SDS-PAGE was slightly modified from that of Poxton *et al.* (1984). Briefly, strains were grown overnight in PPY broth (Holbrook *et al.*, 1977); the washed pellets were extracted with 10 mM EDTA at 45°C for 30 min; proteins (*c.* 100 µg as measured by the method of Lowry *et al.*, 1951), were separated in 10% slab gels at constant current; mol. wt markers (Bio-Rad) were included in electrophoresis runs. Gels were then stained with Coomassie blue, photographed and dried in a gel drier.

Results

Ten patients had *C. difficile*-associated diarrhoea; all were women admitted to the same surgical unit.

Table I. Clinical data of patients with *C. difficile*-associated diarrhoea

Patient no.	Age	Surgical operation	Antibiotic	Result of test for faecal cytotoxin
1	52	cholecystectomy	clindamicin	n.t.
2	51	cholecystectomy	ampicillin	n.t.
3	54	cholecystectomy	ceftriaxone	+
4	40	cholecystectomy	clindamicin	n.t.
5	85	colon carcinoma	ampicillin	—
6	23	pelvic inflammatory disease	cefotaxime	+
7	53	intestinal obstruction	cefotaxime	+
8	66	metastatic carcinoma	cefoxitin	+
9	32	cholecystectomy	clindamicin	+
10	86	cholecystectomy	ceftriaxone	+

n.t. = Not tested.

Table I shows the clinical data of these patients. Their ages were in the range 23–86 years (mean 54). All but two patients (nos. 6 and 8) underwent abdominal surgery, and used bed-pans and had rectal tubes *in situ* during the first and second days after surgery. Antimicrobial prophylaxis was started on the day of operation; patients nos. 6 and 8 received antibiotics on the day of admission. Three patients received clindamycin, two patients ampicillin, two patients ceftriaxone, two cefotaxime and one patient cefoxitin. Intervals between the first antibiotic dose and the start of diarrhoea

were 2–5 days. Faecal cytotoxin assays were done on samples from seven patients and toxin was detected in six. In all patients, symptoms subsided promptly with vancomycin treatment (250 mg four times daily for 5 days).

All environmental samples from patient-related zones and the nurses room gave negative results in cultures for *C. difficile*. The majority of samples obtained from the environment and from items in the sluice room and in the storage room gave positive cultures for *C. difficile* with the exception of the equipment for administering enemas (table II). Samples from the hands of the nurse gave positive cultures for *C. difficile*. Cultures of faecal samples

Table II. Distribution of *C. difficile* in the environment

Sites examined	Number positive/number sampled
Case-related zones	
Floors	0/50
Walls	0/10
Bedlinen (in use)	0/20
Bedside tables	0/10
Chairs, benches	0/10
Nurses room	
Floor	0/1
Furniture	0/7
File	0/2
Wash-basin	0/1
Trolleys	0/3
Sluice room	
Floor	3/3
Sluice	2/2
Bedpans (used)	4/5
Chamberpots (used)	3/5
Washing water	1/1
Shelves	1/4
Storage room	
Floor	3/3
Bedpans (clean)	2/5
Rectal tubes (clean)	2/6
Equipment for enema	0/2

Table III. Results of typing *C. difficile* isolates

Strains tested	Antibiotic susceptibility	Electrophoretic type
Patient		
1	R	II
2	R	II
3	R	II
4	R	II
5	R	II
6	R	II
7	R	II
8	R	II
9	S	VII
10	R	I
Environment		
sluice	R	II
floor of sluice room	R	II
chamber pots	R	II
rectal tubes	R	II
bedpans	S	VII
bedpan washing water	R	I
Hands of nurse	R	II

R = resistant to the antibiotics tested.

S = susceptible to the antibiotics tested.

We observed a cluster of *C. difficile*-associated diarrhoea in a surgical unit and the contamination of the environment with the same micro-organism. By SDS-PAGE we were able to recognise a protein pattern common to the majority of the strains isolated from patients and the environment; only four strains had different protein profiles. If unselected *C. difficile* strains from various sources are examined, several different patterns can be obtained by this method (Pantosti *et al.*, 1986). Therefore, our results suggest the circulation of the

same "epidemic" strain in the hospital ward. The protein pattern of this strain was identified as type II.

By antibiotic susceptibility testing only two strains could be identified as different from the "epidemic" resistant strains: the usefulness of this method is limited by the relatively uniform susceptibility-pattern of *C. difficile* strains to antibiotics (Gianfrilli *et al.*, 1984).

We did not find *C. difficile* in the areas of patient beds but we isolated it from the hands of the nurse who attended the patients, from the sluice room and dirty items, and from clean medical equipment kept in the storage room. It is likely, therefore, that spread of infection among the patients occurred via the hands of the nurse and the contaminated fomites.

All patients involved in this hospital outbreak had one or more risk factors for developing *C. difficile*-associate diarrhoea—age, previous antimicrobial therapy and abdominal surgery, all factors known to alter the bowel flora.

The number of new cases of *C. difficile*-associated diarrhoea gradually diminished after the contaminated rooms were thoroughly cleaned with sporicidal disinfectant (glutaraldehyde), disposable equipment was used whenever possible, and frequent and effective hand washing was encouraged. No isolation of patients was instituted, in contrast to reports in other *C. difficile* outbreaks (Heard *et al.*, 1986). These simple measures appeared sufficient to control the spread of *C. difficile* in the hospital ward and, therefore, their routine application could prevent similar episodes.

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