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Clostridium difficile-associated diarrhoea: epidemiological data from Western Australia

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SUMMARY

The incidence of *Clostridium difficile*-associated diarrhoea (CDAD) was investigated retrospectively at a 690-bed teaching hospital for the period 1983–92. Our aims were to determine: (i) the distribution by age and sex of patients with CDAD, (ii) the possibility of a seasonal trend and, (iii) the influence of infection control procedures, contamination of the hospital environment and the use of third-generation cephalosporins. The laboratory diagnosis of CDAD was based on demonstration of the organism by stool culture and/or detection of specific cytotoxin in stool filtrates. *C. difficile* was detected in 917 patients who were being investigated for diarrhoeal illness. Yearly isolations varied from a low of 49 in 1983 to a high of 120 in 1990 (Chi square for linear trend 128·8; $P < 0{\cdot}005$). Most patients were elderly, with 63% aged 60 years or more; the majority (59%) were female. The relationship between culture of *C. difficile* and detection of cytotoxin in faecal extracts was also examined. Sixty percent of a sample of 132 isolates from patients in whom faecal cytotoxin was not detected produced cytotoxin *in vitro*, suggesting that culture is a more sensitive indicator of infection with *C. difficile* than cytotoxin detection. When the total number of faecal specimens received in the laboratory was used as a denominator there was an increase in the number of incident cases of CDAD between 1983 and 1990, apart from 1986. When occupied bed days was used as the denominator a similar trend was observed with a peak in 1990. These increases correlated with an increase in the use of third-generation cephalosporins at SCGH between 1983 and 1989 (Pearson's correlation coefficient, 0·90). The introduction of Body Substance Isolation in 1989, in conjunction with other infection control procedures, appears to have halted the rise, despite a continuing use of broad-spectrum cephalosporins. In order to reduce the number

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of cases of CDAD, either a reduction in levels of environmental contamination or a reduction in the use of third-generation cephalosporins is required. If this can be achieved the economic consequences, in terms of an opportunity cost, will be considerable.

INTRODUCTION

Clostridium difficile is the causative agent of pseudodomembranous colitis (PMC) and the major aetiological agent of antibiotic-associated diarrhoea (AAD) [1]. Nosocomial acquisition of *C. difficile* has been demonstrated [2] and outbreaks of diarrhoea associated with *C. difficile* have been reported in hospital patients [3]. *C. difficile* is the most common cause of diarrhoea among all patients treated at Sir Charles Gairdner Hospital (SCGH), a 690-bed adult teaching hospital in Perth, Western Australia [4]. A number of factors predispose individuals to infection with *C. difficile*. The two most important risk factors are exposure to the organism and exposure to antibiotics, particular third-generation cephalosporins [5, 6]. Contamination of the hospital environment by *C. difficile* spores contributes to the spread and persistence of *C. difficile* within institutions [7].

The laboratory diagnosis of *C. difficile*-associated diarrhoea (CDAD) is based on demonstration of the organism by stool culture and/or detection of specific cytotoxin/enterotoxin in stool filtrates [8]. Methods for the laboratory diagnosis of CDAD at SCGH have not altered since 1983 and this has allowed us to compare yearly isolation rates for *C. difficile* and other gastrointestinal pathogens over a 10-year period. The aims of our investigation were to determine: (i) the distribution by age and sex of patients with CDAD, (ii) the possibility of a seasonal trend and (iii) the effect of contamination of the hospital environment and the influence of infection control procedures like Body Substance Isolation (BSI). BSI is a system of infection precautions intended to reduce nosocomial transmission of infectious agents among patients [9]. A number of studies have suggested that unless cytotoxin is present in the stool, isolation of the organism alone is not indicative of disease caused by *C. difficile* [10]. A fourth aim therefore was to examine the relationship between stool cytotoxin results, culture results and the production of cytotoxin by organisms *in vitro*. Finally, we examined the relationship between the use of third-generation cephalosporins and the incidence of CDAD.

MATERIALS AND METHODS

Faecal samples submitted to the Department of Clinical Microbiology were examined for *C. difficile* if they fulfilled one or more of the following criteria: (i) stools were loose or water; (ii) red or white cells were present on microscopy; (iii) there was a history of antibiotic use; (iv) there was a history of inflammatory bowel disease [11].

The methods employed for the isolation of *C. difficile* and other enteric pathogens, and the detection of *C. difficile* cytotoxin in VERO cells, have been described previously [4]. They included the use of a selective enrichment broth for *C. difficile* containing gentamicin 5 mg/l, cycloserine 250 mg/l and cefoxitin 8 mg/l (GCC broth) and culturing on cycloserine-cefoxitin fructose agar (CCFA) [12]. Screening of GCC broths and final identification of *C. difficile* were performed

using a commercially available latex particle agglutination test (Mercia Diagnostics Ltd, Guildford, Surrey) [13].

Patients from whom *C. difficile* and other gastrointestinal pathogens were isolated, or *C. difficile* cytotoxin detected, were identified by examining the Department of Clinical Microbiology's laboratory records between the years 1983 and 1992. When information was missing from laboratory records, in-patient medical records were checked. The total number of faecal specimens processed in the laboratory in each year was also determined from laboratory records. This allowed us to calculate the incidence of infection due to *C. difficile* and other gastrointestinal pathogens as a proportion of specimens processed. In a similar manner occupied bed days (OBD) were also used as a denominator. OBD were defined as: the arithmetic difference in days between the 'in event date' (admission, transfer-in) and the 'out event date' (separation, transfer-out), including patient leave days. OBD for 'one day stay patients' were calculated as one. Data on the number of discharges from SCGH by age and sex were available for the period 1986–92.

Hospital usage of the third-generation cephalosporins, ceftriaxone, cefotaxime and ceftazidime, between 1983 and 1992 was determined by obtaining the amount in grams of each antibiotic dispensed from the hospital pharmacy in one year.

To determine the proportion of cases where *C. difficile* cytotoxin was not detected in stool but a cytotoxigenic strain was isolated, 132 strains of *C. difficile* isolated from patients with a negative faecal cytotoxin assay between 1986 and 1989 were studied further. These isolates were tested for cytotoxin production *in vitro* in the following manner. Each isolate was inoculated into a 5 ml prereduced supplemented brain heart infusion broth and incubated anaerobically at 37 °C for 72 h. Sterile filtrates of the cultures were examined for cytotoxin production in VERO cells as described previously [4] and the results compared to those obtained for faecal cytotoxin.

RESULTS

A total of 917 patients was identified as having been infected with *C. difficile* at SCGH between January 1983 and December 1992. Demographic details were available on 888 (97%) of the 917 patients with CDAD. Figure 1 shows the distribution by age and sex of these patients. Most patients were in the older age groups; 63% were over 60 years, but all age groups were represented. The most frequently represented age group was 70–79 years and nearly 25% of patients came from this group.

Fifty-nine percent (528) of the 888 patients with CDAD were females. In the 70 years and older age groups there was a strikingly increased ratio of females to males compared to other age groups. The ratios of female to male patients with CDAD in the various age groups were as follows: 19 years or less, 0·3:1; 20–29 years, 1·1:1; 30–39 years, 1·6:1; 40–49 years, 1·6:1; 50–59 years, 1·1:1; 60–69 years, 1·2:1; 70–79 years, 1·7:1; 80 years or more, 2·2:1. Using discharge data for the period 1986–92, the average age specific ratios of female to male patients in hospital were calculated as follows: 19 years or less, 0·8:1; 20–29 years, 0·9:1; 30–39 years, 0·9:1; 40–49 years, 1·1:1; 50–59 years, 0·9:1; 60–69 years, 0·8:1; 70–79 years, 0·9:1; 80 years or more, 1·7:1.

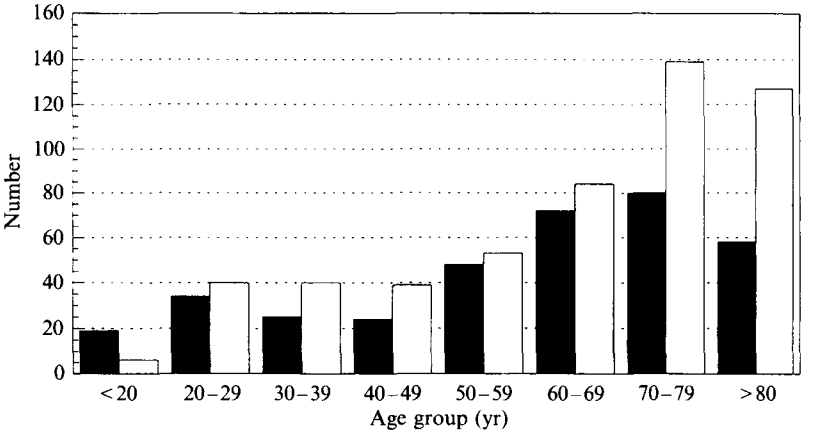

Fig. 1. *Clostridium difficile*-associated diarrhoea at SCGH by age and sex, 1983–92. ■, Male; □, female.

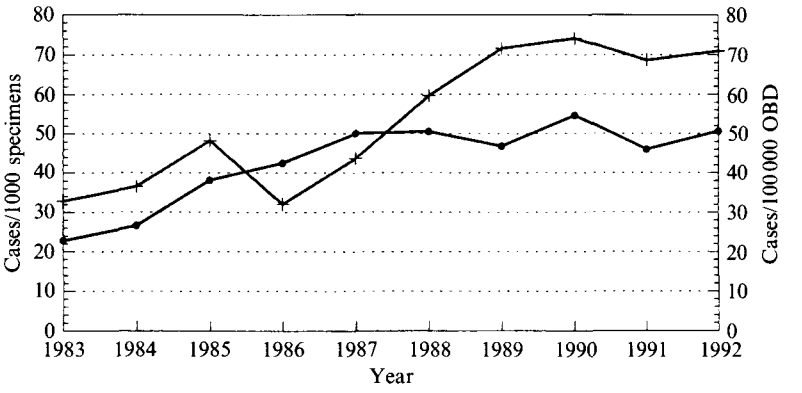

Fig. 2. Incidence of *Clostridium difficile*-associated diarrhoea at SCGH, 1983–92. ●——●, Cases/100000 OBD; +——+, cases/1000 specimens

The number of patients infected each year with *C. difficile* ranged from a low of 49 patients in 1983 to a high of 120 patients in 1990. When the total number of faecal specimens processed in the laboratory was used as the denominator, there was an increase in the proportion of positives from 1983 onwards (except for 1986), reaching a peak in 1990 (Fig. 2). When OBD were used as a denominator a similar trend was observed, with the isolation rate also peaking in 1990. BSI and improved infection control procedures were instituted in 1989 and, since 1990, the isolation rate, by both specimen number and OBD, has remained steady.

While isolation rates for *C. difficile* continued to rise until 1990 (Chi square for linear trend 128·765; $P < 0{\cdot}005$), those for other gastrointestinal pathogens fluctuated with no obvious trend (Table 1). A statistically significant seasonal variation in the isolation rate for *C. difficile* could not be demonstrated.

Figure 3 shows the total usage in grams of third generation cephalosporins at SCGH over the last 10 years. Apart from 1988 there was a steady increase in the amounts of these agents used until 1989, when a plateau was reached. Another

Table 1. *Isolation rates for* Clostridium difficile *and other gastrointestinal pathogens at SCGH per 1000 specimens processed*

Organism	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992
Clostridium difficile	32·8	36·5	48·2	32·0	43·7	59·7	71·6	74·1	68·6	70·9
Salmonella spp.	5·3	5·1	4·1	2·7	3·3	4·3	7·0	5·5	2·7	3·3
Shigella spp.	2·0	0	1·7	1·4	0·4	3·8	1·4	1·8	0	0·6
Campylobacter spp.	7·3	8·9	12·9	4·5	8·8	8·2	13·3	19·1	13·8	13·2
Parasites	18·1	25·6	20·0	11·4	8·4	22·4	14·0	12·3	11·7	10·5
Total specimens	1493	1558	1700	2874	2378	1827	1424	1620	1444	1510

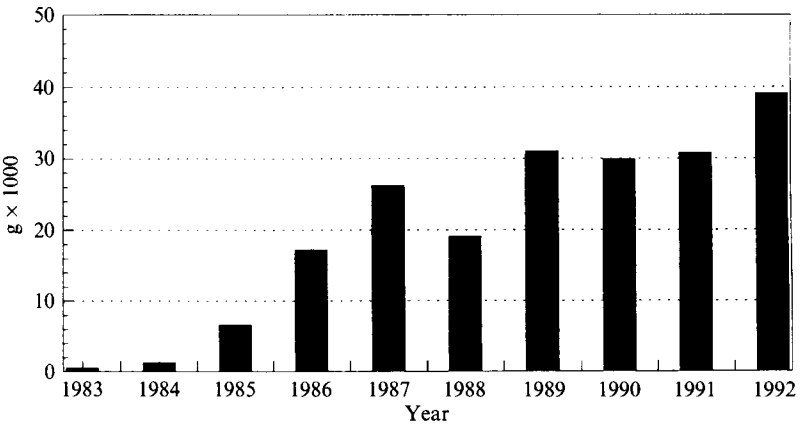

Fig. 3. Third-generation cephalosporin usage at SCGH, 1983–92.

substantial increase was recorded in 1992. The correlation coefficient between grams of third-generation cephalosporins used at SCGH over the last 10 years and numbers of cases of *C. difficile* infection was 0·90 (Pearson's correlation coefficient) indicating a strong relationship.

The relationship between culture of *C. difficile* and detection of cytotoxin in faecal extracts was also examined. Of the total number of patients in whom *C. difficile* was detected, 47·4% were positive by both culture and faecal cytotoxin, 46·4% were positive by culture but negative by faecal cytotoxin and 6·2% were negative by culture but positive by faecal cytotoxin. When isolates from 132 patients were tested for cytotoxin production, 60% of isolates which had been recovered in the absence of faecal cytotoxin produced cytotoxin *in vitro*.

DISCUSSION

The role of *C. difficile* in gastrointestinal infections has received attention only in the last 15 years. Although it was thought initially that *C. difficile* was the aetiological agent only for relatively rare cases of PMC, it is now apparent that *C. difficile* can be responsible for a wide spectrum of disease. These may range from

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mild, self-limiting diarrhoea to severe AAD and fulminant PMC. The increasing importance of *C. difficile* as a cause of nosocomial diarrhoea in hospital patients prompted a retrospective analysis of our institution's laboratory records for a 10-year period. Some interesting and, to our knowledge, previously undocumented trends became apparent.

Most of our isolations of *C. difficile* came from elderly patients, a pattern similar to that reported by others [14]. This was not an unexpected finding as previous investigators have noted a decrease in humoral immunity to *C. difficile* infection corresponding to an increase in age [15]. Another important feature was the predominance of female patients, a fact only recently highlighted in the literature [16]. Fifty-nine percent of our patients were female. This was a real difference as there were approximately equal numbers of male and female patients in the hospital during the study period. It is interesting to speculate on reasons for the apparently greater susceptibility of female patients to infection with *C. difficile*. One possible explanation may be related to antibiotics prescribed for urinary tract infections, particularly in older females, leading to a greater predisposition to infection with *C. difficile*.

Diagnosis of infection with *C. difficile* at SCGH involves both culture of the organism and detection of cytotoxin (toxin B) in faecal specimens. We found that 47% of patients with *C. difficile* were both culture and faecal cytotoxin positive. However, an almost equal number of patients were positive by culture but had no detectable specific cytotoxin in stool. A number of studies have suggested that unless cytotoxin is present in the stool, isolation of the organism alone is not indicative of disease caused by *C. difficile* [10]. We therefore retrieved 132 isolates of *C. difficile* obtained from patients at SCGH and tested them for cytotoxin production *in vitro*. These results were compared to the faecal cytotoxin result from the stool specimen from which the isolate was obtained. Sixty percent of isolates from specimens with a negative cytotoxin assay produced cytotoxin *in vitro*, indicating that a negative faecal cytotoxin result cannot exclude the presence of a toxigenic strain of *C. difficile*. Hence faecal cytotoxin testing may not be as reliable an indicator of infection as some studies suggest. Furthermore, the recent case report of a non-cytotoxigenic (but enterotoxigenic) strain of *C. difficile* causing a confirmed case of PMC [17] also supports the conclusion that culture may be a more sensitive and reliable indicator of infection with *C. difficile*.

The increasing incidence of CDAD at SCGH over the last 10 years also appears to be real. When two different denominators, number of faecal specimens processed in the laboratory and OBD, were used to calculate incidence rates, there was an increase in the incidence of CDAD, using both denominators, over the study period. The only marked deviation in this trend was in 1986, when the number of cases of CDAD per 1000 specimens fell. However, during that year the hospital experienced an outbreak of infection with gentamicin-resistant Enterobacteriaceae and the number of faecal specimens processed by the laboratory increased markedly as routine screening procedures were instituted.

Two factors may be particularly important in the rapid rise in infection with *C. difficile*. First, contamination of the hospital environment with *C. difficile* presents a significant problem. The environment is an important reservoir for *C. difficile* and recent studies indicate that most patients acquire the organism from an

exogenous source [2, 18]. Environmental sampling at SCGH undertaken in 1990–1 showed that 38 (17·5%) of 217 sites sampled were contaminated with *C. difficile* (G. L. O'Neill and T. V. Riley, unpublished results). Particularly heavy contamination was found in rooms that harboured a patient infected with *C. difficile*, either at the time of sampling or within 1 month of discharge. These results suggest that there is a significant reservoir of *C. difficile* in the environment at SCGH.

Second, the inappropriate use of certain broad-spectrum antibiotics may predispose more patients to infection with *C. difficile* at SCGH. Treatment with third-generation cephalosporins is a risk factor for the development of CDAD, both at our institution [6] and elsewhere [5]. The increase in incidence of CDAD at SCGH over the last 10 years paralleled the substantial and increasing amounts of third-generation cephalosporins used. Investigations are currently underway to examine this relationship in more detail.

The relative importance of these two factors is difficult to assess; however, it is likely that they are both significant and a reduction in either risk factor should lead to a reduction in disease. From our data there is evidence that the incidence of CDAD at SCGH has not increased from 1990 onward. This stabilization occurred following the introduction of BSI in 1989 and it is tempting to speculate that this may be the reason for the reduction in incidence. Other investigators have demonstrated a significant reduction in CDAD following the introduction of various infection control procedures such as the mandatory use of vinyl gloves in particular hospital wards [19]. However, given that environmental contamination with *C. difficile* is a major issue relating to CDAD, it is difficult to conclude that improved infection control procedures alone, aimed at reducing person-to-person transmission, will reduce the incidence of CDAD.

It is also apparent from our data that there was no substantial increase in third-generation cephalosporin usage at SCGH for the 3 years 1989–91. It is likely that this fact, together with the change in infection control procedures, has resulted in the slight decline for CDAD from 1990 onward. In order to reduce further the number of cases of CDAD, either a reduction in levels of environmental contamination or a reduction in the usage of third-generation cephalosporins is required. Given the nature of *C. difficile*, particularly its ability to survive for long periods in the environment [7], it will be difficult to achieve the first of these requirements in the short term. It may therefore fall to hospital administrators and microbiologists to help formulate antibiotic policies which do not encourage the proliferation of *C. difficile*.

In conclusion, it is appropriate to highlight the economic consequences of CDAD in hospital patients. If the increased bed stay due to CDAD is 10 days (an underestimate according to some studies [20]) and the average cost of a hospital bed per day is $600 then, for 100 patients per year with CDAD, the cost to the hospital is $600 000 per year. While recognizing that this is a simplistic approach to the economic consequences of CDAD, it nonetheless helps identify an opportunity cost. In those hospitals where CDAD is perceived as a problem, it is therefore worthwhile, purely on economic grounds, to attempt to resolve the problem. In those institutions where *C. difficile* is not a problem, it is certainly economically worthwhile maintaining the *status quo*.

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