Hospital-Acquired *Clostridium difficile* Infection amongst ICU and Burn Patients in Kuwait

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Key Words

Hospital acquisition · Intensive care units · *Clostridium difficile* · Diarrhoea

Abstract

Objectives: To prospectively study the prevalence of nosocomially acquired *Clostridium difficile*, a major cause of diarrhoea in hospitalized patients, in the intensive care units (ICUs) and burn unit (BUs) of three teaching hospitals in Kuwait. **Methods:** During a 1-year prospective study, stool/rectal swabs were obtained from 344 patients admitted into the ICUs of Mubarak Hospital (ICU-1), the Kuwait Cancer Control Centre (ICU-2), and the BU of Ibn Sina Hospital. The presence of *C. difficile* and/or its toxin was detected by serially culturing the specimens on differential, selective and enriched media and the use of TOX-A/B test, on admission and at subsequent 1-weekly interval until discharge. **Results:** Out of the 344 patients admitted into these units, over a study period of 1 year, only 263 (77%) were evaluable. All of them had negative stool culture/toxin on admission. Overall, 25 (9.5%) of these 263 patients acquired *C. difficile* during their hospitalization. Thirteen (7%) of 187 patients acquired *C. difficile* in ICU-1, 9 (36%) of 25 on

ICU-2 and 3 (5.9%) of 51 patients in BU. Eight (32%) developed diarrhoea attributable only to *C. difficile* and/or toxin, and the remaining 17 (68%) were asymptomatic: none had pseudomembranous colitis. The diarrhoea in these patients was associated with antibiotic use, the main trigger-antibiotics being the third-generation cephalosporins. Acquisition occurred within 4–53 days of admission, with the majority occurring in the first 15 days. **Conclusion:** Overall, the prevalence of hospital-acquired *C. difficile* infection/colonization was less than 10%. The use of third-generation cephalosporins was high and was related to the development of diarrhoea. Once acquired, diarrhoea developed in about one third of *C. difficile*-positive cases, an indication that *C. difficile* infection/colonization endemic in the hospital ICUs studied is usually transmitted among the hospitalized patients.

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Introduction

Clostridium difficile often causes a spectrum of disease referred to as *C. difficile*-associated disease (CDAD), which ranges from mild, self-limiting diarrhoea to poten-

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tially life-threatening pseudomembranous colitis (PMC) [1–6]. This organism has been associated with outbreaks of diarrhoea and colitis in adult patients receiving antibiotic therapy during hospital admission [7–11]. Person-to-person transmission on hospital wards, particularly geriatric wards, as well as environmental contamination and carriage on the hands of hospital workers have been documented [12–18]. Hospital-acquired infections, in general, occur in 5–10% of patients admitted to hospitals with consequent increase in morbidity, mortality and cost. The majority of these infections are acquired in the intensive care units (ICUs) [19]. Even though *C. difficile* is the most frequent hospital-acquired enteric pathogen isolated from hospitalized adult patients [18, 20] and has also become a problem for outpatient and extended care facilities [21, 22], little is known about its acquisition and role in nosocomial diarrhoea in the ICU and burn unit (BU) settings.

As far as we know, such an important pathogen has not been studied in the ICU and BU settings of large hospitals like ours, which lack general enforceable guidelines on the use of antibiotics and where their use sometimes defies rationalization. This study was designed to investigate the acquisition of *C. difficile* by patients admitted into the critical care units (ICU and BU) of three of our hospitals.

Material and Methods

Patients and Samples

A total of 187, 51 and 25 consecutive patients admitted to special hospital units, ICUs and BU, of Mubarak Al-Kabeer Hospital (ICU-1), Ibn Sina Hospital (BU) and Kuwait Cancer Control Centre (ICU-2), respectively, were studied over a period of 1 year, July 1999–June 2000. Freshly passed stool (rectal swabs, in Amies transport medium, if collection of stool was not feasible) was taken from each patient for culture on the day of admission and weekly thereafter until the patient was discharged or until diarrhoea secondary to *C. difficile* developed. The number of bowel motions was recorded daily to note when a patient had developed diarrhoea. *C. difficile*-associated diarrhoea was defined as: (i) a minimum of six loose bowel motions in 36 h; (ii) a positive stool culture and/or cytotoxin assay for *C. difficile* and/or endoscopic evidence of PMC, and (iii) no other explanation for the presence of the diarrhoea [23]. Mucosal biopsy of the sigmoid colon and other appropriate parts of the colon was taken during endoscopic examination of a suspected clinical case of PMC. General patient demographic bio-data, including name, hospital number, room and bed location, previous hospital admissions, age, sex, underlying diseases, different classes of antibiotic administered, were all recorded on a protocol data sheet. Five of the 8 patients with CDAD were on ceftriaxone combined with amikacin, 2 were on ampicillin plus amikacin and 1 received metronidazole plus ceftazidime.

Other Samples

Hospital Personnel. A sample group of doctors, nurses and physio- and respiratory therapists caring for patients with positive *C. difficile* cultures in the ICU and BU were investigated before and after making contact with the patients. Their samples were taken by making hand-surface imprints and fingernail impressions on selective *C. difficile* agar.

Transportation of Specimens

Stool Samples. All stool/rectal swab samples were transported to the Anaerobe Research Laboratory in the Department of Microbiology, Faculty of Medicine, without any delay. Samples from Ibn Sina Hospital and Kuwait Cancer Control Centre (both 16 km away) were sent immediately through designated porter. Where samples could not be sent immediately they were kept in the refrigerator or frozen and then sent within 24–48 h. This method has been shown to preserve the toxins in the stool samples [24].

Biopsy Specimens. Rectal/colonic biopsies were taken by the gastroenterologist and sent in formalin immediately to the Department of Pathology for histopathological studies.

Culture Media

Selective and non-selective media were used throughout for the isolation of *C. difficile* and other enteric pathogens. These were fastidious anaerobe agar (Lab M, Bury, UK), cycloserine-cefoxitin egg-yolk agar (CCEYA, Lab M) plus 6% horse blood, Robertson cooked meat medium containing 25 ml of fastidious anaerobe broth (Lab M), Brucella agar (Acumedia, Baltimore, Md., USA) plus 6% horse blood and anaerobe identification medium (Lab M). Non-clostridial pathogens capable of causing diarrhoea were investigated using MacConkey agar (Unipath, Basingstoke, England), SS agar (Unipath), XLD agar (Unipath), and TSI slants.

Inoculation and Isolation

Stool or rectal swabs were inoculated onto the various selective and enriched media, within 20 min of receipt in the laboratory. The process was carried out inside an anaerobic cabinet (Ruskin Tech. Ltd., Guiseley, Leeds, UK) and incubated for 48 h at 35 °C. Agar plates for non-clostridial pathogens were incubated in an ordinary incubator, at 37 °C for 24 h. Environmental and personnel samples were plated onto *C. difficile*-selective media only. To facilitate the detection of low concentrations of *C. difficile* and to stimulate spore germination, pre-reduced peptone broth supplemented with cefoxitin (39 µg/ml) and 0.1% sodium taurocholate (98% pure; Sigma Chemical, St. Louis, Mo., USA) were inoculated concurrently with the *C. difficile*-selective agar as previously described [25].

Identification

Colonies of putative *C. difficile* usually fluoresce a yellow-green colour under long-wave ultraviolet illuminator; therefore, suspected colonies on the CCEYA were exposed to long-wave ultraviolet light (565 nm). Representative colonies with characteristic smell and fluorescence yellowish-green under UV light were identified by API 20A (bioMérieux, France).

Toxin Detection

All strains were tested for toxin A/B (enterotoxin) production with the *C. difficile* TOX-A/B test, an enzyme immunoassay (ELISA), for the rapid detection of toxin A and B (Tech Lab, VPI Research Park, Blacksburg, Va., USA), a procedure carried out

Table 1. General characteristics of hospital-acquired *C. difficile* culture-positive patients in the ICUs/BU

Characteristics	Culture-positive patients		
	ICU-1 (n = 13)	BU (n = 3)	ICU-2 (n = 9)
Age, years			
Range	0.29–65	1.0–1.5	16–60
Mean	46.2	1.2	34.8
Days in the units, mean	27.7	28.0	21.0
Days before culture-positive, mean	14.6	13.2	51.2
Patients on antibiotics	10 (77)	3 (100)	9 (100)
Patients on multiple antibiotics	6 (46)	3 (100)	9 (100)
Underlying illness			
Cardiovascular	4 (31)	0	0
Diabetes mellitus	2 (15)	0	0
Renal insufficiency	1 (8)	0	0
Burn	0	3 (100)	0
Malignancy	0	0	9 (100)
Respiratory failure	6 (46)	0	0
Previous history of CDAD	0	0	0
Patients with diarrhoea	4 (31)	0	2 (22)
Patients symptom-free	9 (69)	3 (100)	7 (77.8)
Percentage is given in parentheses.			

according to the manufacturer's package insert. This ELISA test was also used to detect *C. difficile* antitoxin in faecal samples of all patients; it has been shown to be as sensitive and specific as the tissue culture assay method regarded as the 'gold standard' for toxin detection.

Statistical Analysis

Statistical analysis of the data generated in this study was done by χ^2 statistical test for univariate comparisons of proportions using SPSS statistical package for Windows.

Results

Prevalence of Nosocomial Acquisition of *C. difficile*

A total of 377 patients were admitted into the three units over a period of 1 year. Out of these, 344 were eligible for investigation, but only 276 (80%) were eventually enrolled. The patients' characteristics are given in table 1. The mean number of days in the units was 25.6 days (range 2–50 days), with 64% staying for 4–10 days, 17% staying for 11–20 days, 10% staying for 21–30 days, 3% for 31–40 days and 6% for over 40 days. Of the 276 patients enrolled, 25 were culture-positive for *C. difficile* after admission. Thus, 9% of the patients who had negative cultures on hospital admission had nosocomial acquisition of *C. difficile*. Analysis by hospital unit showed that

13 (7%) out of 187 patients were positive in Mubarak Al-Kabeer Hospital ICU (ICU-1), 3 (6%) out of 51 in Ibn Sina Hospital (BU) and 9 (36%) of 25 in the Kuwait Cancer Control Centre (ICU-2). There was a significant difference between the acquisition rate in the ICU-2 and ICU-1 or BU ($p < 0.001$). Out of a total of 25, 8 (32%) patients developed diarrhoea attributable only to *C. difficile* and the other 17 (68%) patients were symptom-free.

Time of Acquisition

The culture-positive patients stayed in the units for 4–53 days with a mean length of stay of 13.3 days. Twelve (48%) of the patients with nosocomial acquisition of *C. difficile* became culture-positive within 4–10 days, 5 (20%) 11–20 days, 3 (12%) 21–30 days, 2 (8%) 31–40 days and 3 (12%) >40 days. Almost half of the nosocomially infected patients acquired the organism within the 1st week of admission, and by the end of 2 weeks, 17 (68%) of those whose culture would become positive had acquired *C. difficile*. Thus, on the basis of time of *C. difficile* acquisition and patients' characteristics, two groups of patients were identified: patients who acquired the organism within 2 weeks of hospital admission (designated as early acquisition) and those who acquired it after 2 weeks of hospitalization (late acquisition).

Table 2. Characteristics of patients with early and late hospital-acquired *C. difficile*

Characteristics	Early acquisition (n = 19)	Late acquisition (n = 6)
Mean age, years	37.6	43.0
Antibiotics	2.3	5.1
Severity of underlying disease		
Mild	0	0
Moderate	5 (26)	0
Severe	11 (58)	4 (67)
Fatal	3 (16)	2 (33)
Cephalosporin		
None	4 (21)	3 (100)
1 week	12 (63)	0
>1 week	3 (16)	0
Nasogastric tube	10 (53)	4 (67)
Endoscopy	3 (16)	1 (17)
Surgery	1 (5)	0

Number and percent of patients (in parentheses).

Table 3. Characteristics of patients with and without diarrhoea

Characteristics	With diarrhoea (n = 8)	Symptom-free (n = 17)
Age, years	61.3	27.6
Antibiotics	2.0	2.5
Patients with toxigenic strain	8 (100)	5 (29)
Patients with non-toxigenic strain	0	12 (71)
Patients with toxin in stool	8 (100)	3 (18)
Patients with AAD	7 (88)	0
Patients with AAC	1 (12)	0

AAD = Antibiotic-associated diarrhoea; AAC = antibiotic-associated colitis.

Percentage is given in parentheses.

Table 2 shows the clinical characteristics of patients with early and late nosocomial acquisition of *C. difficile*. The mean ages in the two groups were almost the same, but the number of antibiotic consumed in the early group was less than half those consumed by those in the late group. Compared with the patients in the early-acquisition group, patients in the late *C. difficile* acquisition group, who had more severe underlying disease and a higher number of deaths, had been exposed to more antibiotics and use of nasogastric tube. However, when cepha-

losporins are considered alone, more patients in the early-acquisition group were exposed to the drugs than in the late-acquisition group.

Prevalence of CDAD

Case Definitions. A patient was defined to have CDAD with at least one positive *C. difficile* assay (culture and/or toxin A/B assay), antibiotic-associated diarrhoea defined as passage of six loose motions in 36 h following exposure to antibiotics and in whom other aetiological causes of diarrhoea had been excluded.

Prevalence of CDAD. Of the 25 *C. difficile* culture-positive patients, 8 (32%) developed CDAD. In table 3, the characteristics of these patients with CDAD and those who were symptom-free are shown. The mean age of those with diarrhoea was 61.3 years compared with 27.6 years of the symptom-free patients ($p < 0.001$). The number of antibiotics consumed by the two groups was about the same. All (100%) of those with diarrhoea were infected exclusively by toxigenic strains compared with only 27% of the symptom-free patients carrying toxigenic strains ($p < 0.05$). *C. difficile* toxin A/B was present in the stool specimens of all the 8 patients with diarrhoea but only in 8% of those symptom-free. Seven (87.5%) of the 8 patients had antibiotic-associated diarrhoea and the remaining 1 had histopathologically proven colitis. The likelihood that diarrhoea would develop was not significantly different between patients with early acquisition (6 of 17; 35.3%) and those with late acquisition (2 of 8; 25%).

Hand Carriage among Healthcare Providers in the Units

The hands of the healthcare providers (nurses, doctors and respiratory therapists) in ICUs and BU were evaluated for the possibility of hand carriage of *C. difficile* as a means of transmission. Samples from the hands of 72 healthcare providers were cultured for *C. difficile* before and after exposure to patients whose cultures were positive. None was positive.

Discussion

The epidemiological study of *C. difficile* is important because of its nosocomial implications and association with antibiotic use. In the three centres, 25 patients acquired *C. difficile*, with an acquisition rate of over 9%; 7% in ICU-1, 6% in BU and 36% in ICU-2. Rates of 12–21% in hospitalized patients on the surgical and medical wards have been reported, with the higher figures repre-

senting periods of *C. difficile* outbreaks [26–28]. The high rate recorded in the ICU-2 was due to an outbreak experienced in the month of June when 8 out of 9 patients were positive for *C. difficile*. It is conceivable that person-to-person spread occurred in the unit presumably via the hands of healthcare providers. However, this would be mere speculation and the sudden surge of *C. difficile* in the unit at this time is difficult to explain without evidence of clonality of the isolates, a task that is now a subject of another investigation. Interestingly, only 2 of the 8 patients with CDAD were from ICU-2. This is, in fact, the more reason why typing all isolates is imperative. It is also noteworthy that all the nosocomial diarrhoea that occurred in ICUs/BU setting, during the 1-year period of study, was due to cytotoxin-producing *C. difficile*. In this study, 8 patients had clinical and bacteriological diagnosis of CDAD: 7 antibiotic-associated diarrhoea and 1 antibiotic-associated colitis.

Four of the 5 children who were culture-positive harboured toxigenic strains without developing diarrhoea. Evidence from various other reports support this finding, showing that children appear to be able to harbour highly toxigenic strains with no evidence of disease [29, 30]. In contrast, all the adults who developed nosocomial diarrhoea in the ICU were infected with toxigenic strains of *C. difficile*.

A positive correlation has been shown between length of stay in the hospital and the acquisition of the organism. Those who would become infected tend to acquire the organism within the first 14 days of admission into the unit (designated as early acquisition) [18]. The vast majority of the patients acquired *C. difficile* early in their stay, particularly in ICU-1 and BU. This observation is supported by an earlier study, reported by McFarland et al. [18], in which early acquisition was also common.

It is well documented that a stable normal flora is inhibitory to the growth of *C. difficile* and predisposition to infection with *C. difficile* is influenced by factors that disrupt the normal gastrointestinal flora of which antibiotics are the major culprit. ICU and BU are two major specialized hospital units where a variety of antibiotics are frequently and freely used more than in any other hospital units. Evidence produced in this study supported the previously held notion that antibiotic usage, particularly the third-generation cephalosporins [31–33], and insertion of nasogastric tube [33] are possible trigger factors for the nosocomial acquisition of *C. difficile*.

In the 7 patients with antibiotic-associated diarrhoea without systemic symptoms, there was no evidence of colitis. It has been postulated, though not proven, that

C. difficile-associated diarrhoea in such cases may be a result of production of a myoelectric factor that produces intestinal motility [34]. The diagnosis of PMC or antibiotic-associated colitis is a pathological one. In the only case of antibiotic-associated colitis diagnosed, various degrees of non-specific colitis were seen with areas of hyperaemia and oedema but no pseudomembrane. The diagnosis of CDAD in our patients was proven by the presence of *C. difficile* toxin A/B in their stool samples. The ultimate laboratory confirmation of the diagnosis rests with the demonstration of toxin in the stool of patients suffering from diarrhoea which cannot be attributed to any other cause.

Conclusion

Most of the previous studies of diarrhoea caused by *C. difficile* conducted during outbreaks have been case-control studies or used clinical laboratory-based cultures of diarrhoeal stools for the identification of patients with positive *C. difficile* cultures. In this study, it was clear that the rate of acquisition of the organism in the units indicates that it is present in these hospital settings and given a trigger factor, e.g. third-generation cephalosporins and insertion of nasogastric tubes, nosocomial diarrhoea can develop in patients admitted into the ICU or BU.

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