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Chapter 4

Clostridium difficile infection in an endemic setting in the Netherlands

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Abstract

The purpose of this investigation was to study risk factors for *Clostridium difficile* infection (CDI) in an endemic setting. In a 34-month prospective case-control study, we compared the risk factors and clinical characteristics of all consecutively diagnosed hospitalized CDI patients (n=93) with those of patients without diarrhoea (n=76) and patients with non-CDI diarrhoea (n=64). The incidence of CDI was 17.5 per 10,000 hospital admissions. *C. difficile* PCR ribotype 014 was the most frequently found type (15.9%), followed by types 078 (12.7%) and 015 (7.9%). Independent risk factors for endemic CDI were the use of second generation cephalosporins, previous hospital admission and previous stay at the intensive care unit. The use of third generation cephalosporins was a risk factor for diarrhoea in general. We found no association of CDI with the use of fluoroquinolones or proton pump inhibitors. The overall 30-day mortality among CDI patients, patients without diarrhoea and patients with non-CDI diarrhoea were 7.5%, 0% and 1.6% respectively. In this endemic setting, risk factors for CDI differed from those in outbreak situations. Some risk factors that have been ascribed to CDI earlier were, in this study, not specific for CDI, but for diarrhoea in general. The 30-day mortality among CDI patients was relatively high.

Introduction

Since 2002, outbreaks caused by *Clostridium difficile* infection (CDI) have been reported in Canada, the USA and Europe, associated with the emergence of a new hypervirulent type. This type has been characterized as North American pulsed-field type 1, restriction-endonuclease analysis group type BI, toxinotype III and PCR ribotype 027 (type 027)[1-5]. During outbreaks in the USA and Canada, the reported incidences of CDI varied between 155 and 225 per 10,000 hospital admissions[3, 6]. Peak incidences of CDI due to type 027 during outbreaks in the Netherlands were remarkably lower, around 50 per 10,000 hospital admissions[7, 8].

Most recent studies on the risk factors of CDI focussed on outbreaks, whereas less is known about CDI in settings with a low incidence. Well described risk factors for CDI in outbreak situations are prior use of antibiotics, increased disease severity, and, in case of outbreaks caused by type 027, advanced age and prior use of fluoroquinolones[9-11].

The aim of our study was to identify risk factors for CDI in a true endemic setting. A second aim was to establish risk factors specific for CDI, in comparison with factors for diarrhoea in general. To answer these questions, we performed a prospective case-control study at the Leiden University Medical Center during a period of 34 months.

Methods

Patients

From July 2006 through April 2009, all hospitalized patients with CDI were included in the study. Tests for CDI were performed daily upon request and on all unformed faecal samples from patients admitted for two days or more, regardless the physicians' request. For each hospitalized CDI patient, two controls were included, matched for ward at which CDI was diagnosed and time of admission. The controls included one control patient without diarrhoea (control patient) and one control patient with diarrhoea and a negative *C. difficile* toxin test (non-CDI patient). Controls were consecutive patients on the alphabetical ward list.

Definitions

Definitions as proposed by the European and American Centres of Disease Control were used[2, 12]. Diarrhoea was defined as ≥3 unformed stools per 24 hours. CDI was

defined as the presence of diarrhoea in combination with a positive toxin test for *C. difficile.* A community association was defined as development of CDI outside the hospital or within 48 hours after admission, without a history of admission in the previous three months. We defined diarrhoea as severe, when it occurred with one or more of the following: bloody stools, hypovolemia, fever (T>38.0^{0}C) and leucocytosis (>12.0x10^9/l), hypo-albuminemia (<20 g/l), pseudomembranous colitis. A complicated course of CDI was defined as: admission to the intensive care unit (ICU), a surgical intervention in association with CDI, or death within one month. Mortality was considered contributable to CDI when a patient died during admission, partly due to the consequences of CDI.

Isolation and characterization of *Clostridium difficile*

C. difficile toxins in stools were detected by VIDAS *C. Diff.* toxin A during the first 12 months of the study and VIDAS toxin A/B assay during the ensuing 22 months (BioMérieux, France). Each positive sample was cultured. Available isolates were identified as *C. difficile* using a PCR to detect the presence of *glu*D and were PCR ribotyped as previously described[8, 13].

Data collection

Approval was obtained from the Medical Review Ethics Committee to collect demographical and clinical patient data. Information was collected on patients' age, sex, co-morbidity, ward of acquisition, disease severity, clinical course and mortality. Furthermore, data were collected on surgery, invasive procedures, admissions, use of antibiotics and other medications in the 3 months prior to CDI. We gathered this information through consultation of the physician in charge, as well as by using patient records and the hospital electronic medical information system. The period of 3 months prior to CDI was determined by calculating backwards from a reference date. For CDI and non-CDI patients, this reference date was defined as the day on which the diarrhoea started. The reference date for control patients was determined by adding the hospitalized period of the matched CDI patient (time between admission and start of diarrhoea) to the admission date of the control patient. Co-morbidity was assessed by both the Charlson co-morbidity index and the ICD-10 classification in ten disease groups; mentioned in table 1[14].

Table 1. Baseline characteristics of patients with CDI, patients with non-CDI diarrhea and control patients.

Risk factors	CDI patients (N=93)*		Non-CDI patients (N=64)**		Control patients (N=76)***	
	N	(%)	N	(%)	N	(%)
Age > 65 years	33	(35.5)	18	(28.1)	18	(23.7)
Male sex	56	(60.2)	32	(50.0)	41	(53.9)
Charlson co-morbidity index						
0	14	(15.1)	12	(18.8)	19	(25.0)
1-2	38	(40.9)	26	(40.6)	32	(42.1)
3-4	28	(30.1)	15	(23.4)	18	(23.7)
5+	13	(14.0)	11	(17.2)	7	(9.2)
Any underlying disease	90	(96.8)	61	(95.3)	70	(92.1)
Malignancy	24	(26.1)	18	(28.1)	21	(27.6)
Solid tumor	10	(10.9)	5	(7.8)	11	(14.5)
Hematologic malignancy	15	(16.1)	13	(20.3)	10	(13.2)
Endocrine diseases	26	(28.0)	16	(25.0)	20	(26.3)
Respiratory tract diseases	14	(15.1)	9	(14.1)	8	(10.5)
Gastro-intesinal tract diseases	36	(38.7)	16	(25.0)	21	(27.6)
Cardiovascular tract diseases	42	(45.2)	27	(42.2)	30	(39.5)
Urogenital tract diseases	42	(45.2)	21	(32.8)	24	(31.6)
Nervous system diseases	6	(6.5)	4	(6.2)	6	(7.9)
Infectious diseases	13	(14.3)	6	(9.4)	7	(9.2)
Muscular / conn. tissue diseases	10	(10.8)	4	(6.2)	7	(9.2)
Other diseases	36	(39.1)	24	(37.5)	22	(28.9)
Any antibiotic	87	(93.5)	48	(75.0)	51	(68.0)
Proton pump inhibitors	64	(68.8)	36	(56.2)	38	(50.0)
NSAIDs	11	(11.8)	3	(4.7)	7	(9.2)
Immunosuppressive agents	54	(58.8)	38	(59.4)	34	(44.7)
Cytostatic agents	21	(22.6)	13	(20.3)	11	(14.5)
Nasogastric tube	39	(44.3)	29	(45.3)	20	(28.2)
Abdominal surgery	35	(37.6)	24	(37.5)	20	(28.6)
Endoscopy	28	(31.5)	16	(25.0)	10	(13.2)
Previous admission	68	(74.7)	19	(30.2)	30	(41.7)
Previous admission to ICU	26	(28.0)	12	(18.8)	5	(6.6)

* N between 88 and 93.
** N between 62 and 64.
*** N between 71 and 76.
ICU: intensive care unit.
NSAIDs: non-steroidal anti-inflammatory drugs.

Statistical analysis

Continuous data were compared between groups using the T-test. The Pearson's-chi-square test and the Fisher's exact test were used for the analysis of proportions. Factors that were associated in univariate analysis (UVA) with a p-value <0.10, as well as putative risk factors from earlier studies, were analyzed in a multivariable model. Here, associations were always adjusted for age, sex, ward and Charlson co-morbidity index. To evaluate the effect of medications and interventions on (CDI) diarrhoea, we performed additional adjustments for co-medication and other interventions. When comparing non-CDI patients with control patients, we also corrected for the time between admission and the reference date. Relative risks were estimated as odds ratios (OR) and presented with a 95% confidence interval (95% CI). Statistical significance was reached with a 2-sided p-value <0.05; trends were defined by a p-value <0.10. All analyses were performed using the SPSS for Windows software package, version 17.0.

Results

During the 34 month study period, 93 patients were diagnosed with CDI. The incidence varied from 0 to 43 per 10,000 hospital admissions with an average of 17.5. During this period, no outbreaks were observed. CDI was community-associated in four patients (4.3%). Most patients (n=30; 32.3%) were hospitalized at the department of internal medicine, followed by the general surgery ward (n=15; 16.1%). Eighty-nine CDI patients were positive on both toxin testing and culture (95.7%). Isolates from 63 (67.7%) patients were available for PCR ribotyping: type 014 was the most frequently found type (n=10; 15.9%), followed by types 078 (n=8; 12.7%) and 015 (n=5; 7.9%). Type 027 was not present. Three patients with CDI had a co-infection with an enterovirus, norovirus, and *Cryptosporidium*, respectively.

The 93 CDI patients were compared to 76 control patients and 64 patients with non-CDI diarrhoea. Of all patients, physicians responded and records were available, however, in some cases (the exact number is depicted in the subscript of table 1) no information about use of nasogastric intubation, surgery or endoscopy was noted.

In the group of non-CDI patients, two patients were diagnosed with a rotavirus and *Giardia Lamblia,* respectively. Among the other 62 patients no causal agent was found. CDI patients had a median age of 56 years; non-CDI diarrhoea and control patients had a median age of 50 years. Of the CDI patients, 60% were male, compared to 50% and 54% of the non-CDI and control patients, respectively. The time span

between admission and start of diarrhoea did not significantly differ between CDI and non-CDI patients.

Characteristics and risk factors

We present baseline characteristics and risk factors for CDI and non-CDI diarrhoea in tables 1 and 2. The use of antibiotics as a risk factor for CDI and non-CDI is depicted in table 3. All following results reached statistical significance in multivariable analysis (MVA), unless otherwise stated.

Age. Patients with CDI were older than control patients (age > 65 years in 35.5% vs. 23.7%; trend in MVA).

Comorbidity. Both CDI and non-CDI diarrhoeal patients had a higher Charlson co-morbidity index (index of 3-4 or >5) than control patients (not significant). CDI patients were more likely to have haematological malignancies, diseases of the urogenital tract or other diseases (all trends in MVA). The category 'other diseases' comprised organ transplants in 69.7%.

Use of medications. Compared to control patients, patients with CDI more frequently used antibiotics, specifically second and third generation cephalosporins. CDI patients also more frequently used penicillin and vancomycin (all significant only in UVA). Furthermore, CDI patients used proton pump inhibitors (PPIs) more frequently (significant only in UVA). The use of antacids (17.2% vs. 18.4%; OR 0.68; 95% CI 0.26-1.79) or the combined use of PPIs and antacids (74.2% vs. 59.2%; OR 0.75; 95% CI 0.29-1.95) was not significantly more frequent in patients with CDI in MVA (data not shown in the table).

Compared to control patients, patients with non-CDI diarrhoea more frequently used third generation cephalosporins but less frequently used first generation cephalosporins.

Interventions and admissions. Patients with CDI, compared to control patients, were more frequently admitted in the previous 3 months, either at the hospital or ICU department. They also more frequently had a nasogastric intubation or an endoscopy (significant only in UVA).

Patients with non-CDI diarrhoea more frequently had a nasogastric intubation (significant only in UVA), and were more frequently admitted to the ICU in the previous 3 months (trend in MVA).

Table 2. Crude and adjusted odds ratios (ORs) for the development of CDI and non-CDI diarrhea.

Risk factors	CDI vs. Control		Non-CDI vs. Control	
	Crude odds ratio (95% C.I.)	Adjusted odds ratio (95% C.I.)	Crude odds ratio (95% C.I.)	Adjusted odds ratio (95% C.I.)
Age > 65 years	1.77 (0.90-3.49) *	1.82 (0.92-3.62) *	1.26 (0.59-2.69)	1.17 (0.54-2.55)
Male sex	1.29 (0.70-2.39)	1.30 (0.70-2.43)	0.85 (0.44-1.67)	0.88 (0.45-1.72)
Charlson co-morbidity index				
0	Reference	Reference	Reference	Reference
1-2	1.61 (0.70-3.72)	1.78 (0.73-4.37)	1.29 (0.53-3.13)	1.35 (0.50-3.66)
3-4	2.11 (0.85-5.24)	2.42 (0.87-6.73) *	1.32 (0.49-3.57)	1.32 (0.41-4.30)
5+	2.52 (0.80-7.95)	2.57 (0.76-8.65)	2.49 (0.76-8.19)	3.10 (0.82-11.7) *
Any underlying disease	2.57 (0.62-10.7)	2.45 (0.58-10.4)	1.74 (0.42-7.27)	2.10 (.0.46-9.56)
Hematologic malignancy	1.27 (0.54-3.01)	2.33 (0.86-6.23) *	1.68 (0.68-4.15)	2.19 (0.70-6.88)
Urogenital tract diseases	1.78 (0.95-3.36) *	1.97 (0.97-4.02) *	1.06 (0.52-2.16)	0.99 (0.42-2.34)
Other diseases	1.58 (0.83-3.02)	1.47 (0.72-3.00) *	1.47 (0.73-2.99)	1.41 (0.65-3.07)
Any antibiotic	6.82 (2.62-17.8) **	5.41 (1.79-16.3) **	1.41 (0.67-2.98)	0.99 (0.40-2.42)
Proton pump inhibitors (PPIs)	2.21 (1.18-4.14) **	1.14 (0.51-2.58)	1.29 (0.66-2.51)	1.01 (0.46-2.22)
NSAIDs	1.32 (0.49-3.60)	0.86 (0.27-2.73)	0.49 (0.12-1.96)	0.34 (0.07-1.57)
Immuno-suppressive agents	1.71 (0.93-3.15) *	1.39 (0.64-3.06)	1.81 (0.92-3.54) *	1.44 (0.64-3.22)
Cytostatic agents	1.72 (0.77-3.85)	1.61 (0.61-4.24)	1.51 (0.62-3.64)	1.64 (0.58-4.63)
Nasogastric tube	2.03 (1.04-3.95) **	1.50 (0.66-3.43)	2.11 (1.04-4.31) **	1.77 (0.70-4.50)
Abdominal surgery	1.51 (0.77-2.94)	1.17 (0.56-2.45)	1.50 (0.73-3.10)	1.28 (0.57-2.84)
Endoscopy	3.03 (1.36-6.75) **	2.64 (1.00-6.96) *	2.20 (0.92-5.27) *	2.63 (0.90-7.64) *
Previous admission	4.14 (2.13-8.05) **	4.49 (2.23-9.01) **	0.61 (0.30-1.23)	0.55 (0.26-1.17)
Previous admission to ICU	5.51 (2.00-15.2) **	5.47 (1.95-15.3) **	3.28 (1.09-9.87) **	2.64 (0.83-8.37) *

* Trend detected ($p<0.10$).
** Significant difference ($p<0.05$).
ICU: intensive care unit.
NSAIDs: non-steroidal anti-inflammatory drugs.

Clinical course

Severe diarrhoea was present among 51 hospitalized patients with CDI (58.6%) and 25 patients with non-CDI diarrhoea (39.7%) (OR 2.22; 95% CI 1.14-4.30). No significant differences between CDI and non-CDI diarrhoeal patients were found regarding the frequency of fever (55.6% resp. 43.3%), bloody stools (12.2% resp. 12.9%) or abdominal pain (54.5% resp. 48.2%). CDI patients did however have a higher white blood cell count (≥15 x 10^9/l: 49.9% resp. 30.0%, OR 2.28; 95% CI 1.13-4.59). Most patients with CDI were treated with metronidazole (n=57; 63.3%), two patients (2.2%) were treated with vancomycin and in 27 patients (30.0%) no specific CDI treatment was initiated. The 30-day and 60-day mortality rates are depicted in figure 1. At one month follow-up, a complicated course was observed in 9 CDI patients (10.3%), comprising two colectomies, four ICU admissions due to CDI and seven deaths (7.5%). CDI contributed directly to three of these deaths, but was not the primary cause. One non-CDI patient (1.6%) and none of the control patients died at one month follow-up. No significant association were detected between the severity of the diarrhoea, treatment or outcome.

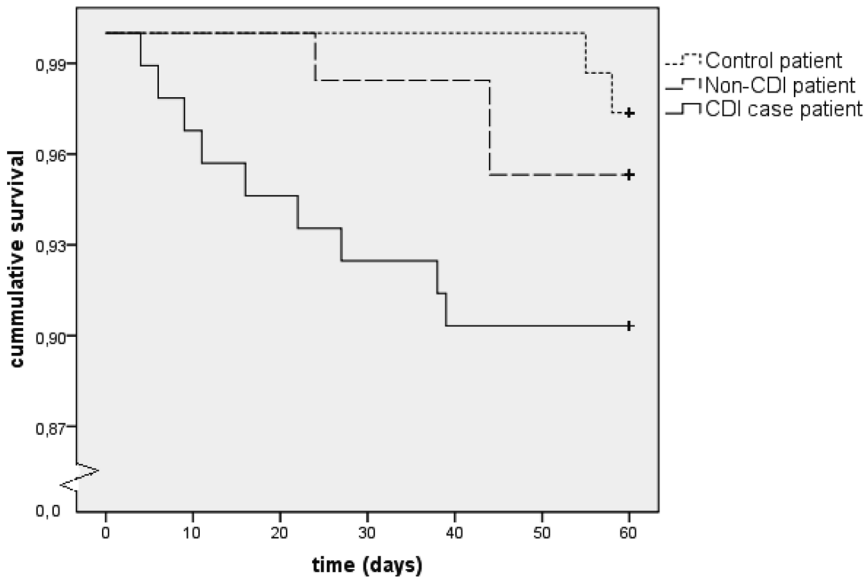

Figure 1. Survival curve of patients with CDI, non-CDI diarrhoea and control patients, in a period of 60 days after the reference date.

Table 3. The use of antibiotics expressed in Defined Daily Doses (DDDs) in patients with CDI and non-CDI diarrhea and control patients.

Antibiotics	CDI patients (N=93)*		Non-CDI patients (N=64)*		Control patients (N=76)*		CDI vs. Control		Non-CDI vs. Control	
	N	(%)	N	(%)	N	(%)	Crude odds ratio (95% C.I.)	Adjusted odds ratio (95% C.I.)	Crude odds ratio (95% C.I.)	Adjusted odds ratio (95% C.I.)
Cephalosporins										
1st generation	13	(14.0)	5	(7.8)	12	(16.0)	0.85 (0.36-2.00)	0.79 (0.21-3.02)	0.45 (0.15-1.34)	0.18 (0.04-0.84) ***
2nd generation	46	(49.5)	20	(31.3)	14	(21.5)	4.26 (2.10-8.67) ***	7.64 (2.42-24.2) ***	1.98 (0.90-4.34)	0.97 (0.31-3.05)
3rd generation	29	(31.2)	12	(18.8)	2	(3.1)	16.5 (3.80-72.1) ***	20.4 (3.50-119) ***	8.42 (1.81-39.2) ***	9.53 (1.66-54.7) ***
Penicillins	51	(54.8)	22	(34.4)	23	(30.7)	2.75 (1.45-5.20) ***	1.47 (0.58-3.72)	1.18 (0.58-2.41)	0.69 (0.27-1.74)
Fluoroquinolones	31	(33.3)	21	(32.8)	20	(26.7)	1.38 (0.70-2.69)	0.57 (0.20-1.62)	1.34 (0.65-2.79)	0.93 (0.32-2.70)
Clindamycin	5	(5.4)	2	(3.1)	1	(1.3)	4.21 (0.48-36.8)	0.75 (0.03-17.2)	2.39 (0.21-27.0)	2.68 (0.14-50.2)
Vancomycin	22	(23.7)	14	(21.9)	7	(9.3)	3.01 (1.21-7.50) ***	0.51 (0.11-2.40)	2.72 (1.02-7.23) ***	1.55 (0.43-5.62)

* This information was known for all patients, except one control patient.
** Trend ($p<0.10$) detected.
*** Significant difference ($p<0.05$) detected.

Discussion

In this 34 months prospective case control study, risk factors for CDI were studied in an endemic setting with a low incidence of CDI. The inclusion of a control group of patients with diarrhoea, tested negative for CDI, enabled us to discriminate between risk factors for CDI and for diarrhoea in general.

Common risk factors for CDI outbreaks, such as age above 65 years and a high comorbidity index, were recognized as trends in our study. This may be due to the fact that these risk factors are of less importance in endemic settings, resulting in a lack of power to discern these risk factors. Other well known risk factors for CDI, such as the use of second generation cephalosporins and previous (ICU) admission were also found in this endemic situation[3, 10, 15]. Conversely, the use of fluoroquinolones or PPIs was not a risk factor for CDI. Furthermore, the previous use of third generation cephalosporins was a risk factor for diarrhoea in general.

The CDI incidence in our hospital was lower than that described in other studies in endemic situations, but comparable to the incidence of 18 per 10,000 hospital admissions found in other Dutch hospitals[16]. Recently, a retrospective study analyzing risk factors for CDI in an endemic setting in USA reported an incidence rate of CDI of 106 per 10,000 hospital admissions, which is a factor 5 higher than what we found in this study[10]. There seems to be a considerable difference, per hospital and per country, in the application of the definition of endemic CDI. Therefore, reported rates of endemic CDI may merely reflect a baseline incidence.

In outbreak situations, the previous use of fluoroquinolones has been recognized as an important risk factor for CDI[9, 11, 17, 18]. This association may be due to disruption of the gut flora by newer fluoroquinolones or the high fluoroquinolone resistance found among hypervirulent type 027 strains[19]. Although fluoroquinolones (mainly ciprofloxacin) were frequently prescribed in this study, we found no association with CDI. An explanation could be that we did not encounter type 027 in our hospital. The most frequently found PCR ribotypes in our study (types 014, 078 and 015) are commonly found in the Netherlands and Europe and are more susceptible to fluoroquinolones than type 027[11].

The use of vancomycin was previously recognized as a risk factor for endemic CDI[10]. Instead, in this study, the association between vancomycin and CDI was strongly confounded by concomitant use of second and especially third generation cephalosporins (the combination is part of the in-house empirical sepsis therapy) and was not a risk factor for CDI.

PPIs raise the gastric pH, which is associated with enhanced bacterial colonization of first part of the gastro-intestinal tract. Studies on the use of PPIs in association with CDI revealed conflicting conclusions[20, 21]. In our study, we found no association of the use of PPIs with CDI. It should be noted that half of the non-CDI and control patients also used PPIs.

Earlier studies have found high contamination and colonization rates with *C. difficile* spores in the hospital environment, among hospitalized patients and asymptomatic carriers[22, 23]. A high colonization pressure on a ward (exposure in time to multiple colonized or infected patients) is associated with an increased risk of CDI [10]. To insure that CDI and control patients were exposed to a similar colonization pressure, we selected control patients from the same ward as CDI patients using the same time period between admission and reference date[24].

We observed contributable and overall mortality of 3.2% and 7.5% after 30-days follow-up, respectively. These proportions are in between the overall 30-day mortality of 4.7%, found in an endemic setting in Canada, and 20% mortality after 60 days in a USA study[25, 26]. These mortality risks are much lower than those reported during outbreaks caused by the type 027 strain[3, 11, 26, 27]. In the Netherlands, a complicated course due to type 027 was described in 12,5%, with an attributable mortality of 6.3%[9].

Our study has several limitations. First, we used the presence of toxins in faeces as a screening test for CDI, which is in agreement with the European recommendations[28]. An alternative standard for diagnosing CDI is the detection of *C. difficile* in faeces by toxinogenic culture or PCR. Application of this definition could have resulted in a different case and non-CDI control group. However, none of the patients with non-CDI diarrhoea developed CDI at a later time during admission, which was in accordance with the high negative predictive value of our toxin test. Second, although the endemic incidence found in our study is comparable to that in other Dutch hospitals, it is lower than incidence rates reported in other studies in endemic situations, which can imply that our findings may not be applicable to endemic situations in other countries[8, 26, 29].

In conclusion, in this endemic setting, some risk factors for CDI were similar to those found in outbreak situations, but some risk factors that have been ascribed to CDI earlier were, in this study, not specific for CDI, but for diarrhoea in general. The use of fluoroquinolones and PPIs did not influence the risk of endemic CDI. CDI patients were more severely ill than non-CDI diarrhoeal patients, as illustrated by a higher leukocyte count and the relatively high 30- and 60-day mortality. Because

CDI is the most important cause of nosocomial diarrhoea, more studies are needed in order to determine the long-term outcome associated with *C. difficile* infections.

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