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Outcomes of *Clostridium difficile*-suspected diarrhea in a French university hospital

Nagham Khanafer^{1,2} · Philippe Vanhems^{1,2} · Frédéric Barbut^{3,4} · Catherine Eckert^{3,4} · Michel Perraud¹ · François Vandenesch⁵ · Christine Luxemburger⁶ · Clarisse Demont⁶ · CDI01 Study Group

Abstract

Clostridium difficile infection (CDI) produces a variety of clinical presentations ranging from mild diarrhea to severe infection with fulminant colitis, septic shock, and death. CDI puts a heavy burden on healthcare systems due to increased morbidity and mortality, and higher costs. We evaluated the clinical impact of CDI in terms of complications and mortality in a French university hospital compared with patients with diarrhea unrelated to CDI. A 3-year prospective, observational, cohort study was conducted in a French university hospital. Inpatients aged 18 years or older with CDI-suspected diarrhea were eligible to participate in the study and were followed for up to 60 days after CDI testing. Among the 945 patients with diarrhea included, 233 had confirmed CDI. Overall, 106 patients (11.2%) developed at least one of the following complications: colectomy, colitis, ileitis/rectitis, ileus, intestinal perforation, megacolon, multiorgan failure, pancolitis, peritonitis, pseudomembranous colitis, renal failure, and sepsis/septic shock. The complication rate was significantly higher in patients with diarrhea related to *C. difficile* than in non-CDI patients (26.6% vs 6.2%, $P < 0.001$). At day 60, 137 (14.5%) patients had died, with 37 deaths among the CDI group (15.9%). Death was attributable to CDI in 15 patients (6.4%). Complications are more frequent among CDI cases than in patients with diarrhea not related to *C. difficile*. Assessment of CDI is necessary to ensure allocation of sufficient resources to CDI prevention.

Keywords *Clostridium difficile* infection · Complication · Prognosis · Recurrence · Ribotype

Introduction

Clostridium difficile is the most common cause of nosocomial diarrhea and a major financial burden for healthcare systems [1, 2]. The epidemiology of *Clostridium difficile* infection (CDI) has changed since the emergence of the 027/NAP1/BI epidemic strain, implicated in large outbreaks and major increases in the incidence and severity of the disease [3, 4]. The clinical presentation of CDI ranges from mild to severe diarrhea, pseudomembranous colitis (PMC), toxic megacolon, intestinal perforation, sepsis, and ultimately death [5]. The pathophysiology for this variation remains poorly understood and mortality rates also vary widely (0.6 to 83%) [6]. The epidemiology of CDI in France is only partially described in a limited number of papers [7, 8]. Consequently, we undertook a prospective cohort study of CDI in a French university hospital to describe the prognosis of patients suffering from *C. difficile*-associated diarrhea. Rates of complications, including mortality, were compared between patients with CDI-associated diarrhea and those with diarrhea unrelated to

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CDI. Polymerase chain reaction (PCR) ribotype was also assessed.

Methods

Study location and patients

Between February 22, 2011 and February 28, 2014, a prospective observational cohort study was conducted in Edouard Herriot Hospital, Hospices Civils de Lyon, a public teaching hospital in Lyon, France (832 beds). Patients aged 18 years or older, hospitalized, and suspected to have CDI were eligible to participate. Outpatients, day-care patients, and patients who refused to participate or were unable to sign the consent form were excluded. Eligible patients who agreed to participate and signed a consent form were included and followed for up to 2 months after CDI testing. The study protocol was approved by the hospital institutional review board (Comité de Protection des Personnes et Comité National Informatique et Liberté) and ethics committee.

Data collection

Patients were actively followed during hospitalization to collect data related to evolution of the diarrheal episode, the occurrence of complications, and death. Data were collected from the clinician in charge of the patient (by phone call), the computerized medical record, the nursing record, and an interview with the patient or a family member during hospital stay or after discharge. For each patient, we completed a standardized form that included information on the following: age, gender, co-morbidities, data on hospital stay, symptoms (e.g., diarrhea, number of stools, fever, abdominal pain, and nausea and vomiting), results of biological and microbiological tests, and infection outcome. After discharge, data were obtained by calling patients and/or their referent physician at days 30 (D30) and 60 (D60).

Microbiological data

C. difficile testing was performed on liquid stool samples after specific request from the physician. Before November 2011, the presence of *C. difficile* was assessed by enzyme immunoassay (EIA, ImmunoCard® Toxins A&B, Meridian Biosciences) of fresh stool samples and by culture on selective media (algorithm 1; Alg1). If the EIA yielded negative toxin results but the culture was positive for *C. difficile*, the toxicity of the isolate was assessed in vitro by toxigenic culture. Between November 2011 and January 2013, a glutamate dehydrogenase (GDH) antigen screening test (ImmunoCard® *C. difficile* GDH assay, Meridian Biosciences) was used first. If positive, stools were subsequently tested for *C. difficile* toxins

A and B by EIA and culture as for Alg1 (Alg2). From February 2013, the laboratory replaced the Alg2 method with a combined immunochromatographic test for GDH and toxins (C. DIFF QUIK CHEK COMPLETE®, Alere) coupled with polymerase chain reaction (PCR, GeneXpert® Systems, Cepheid). If the screening test was positive and the toxin tests were negative, PCR was performed as a confirmatory test (Alg3).

Characterization of isolates was performed using an in-house multiplex PCR assay that detected *tpi*, *tcda*, *tcdb*, *tcde*, *cdtA*, and *cdtB* genes coding for triose phosphate isomerase, toxin A, toxin B, TcdC, and the two components of the binary toxin, respectively. The PCR ribotype was assessed by capillary gel electrophoresis. Primer sequences were described by Bidet et al. [9], labeled at the 5' end with fluorochrome. Banding patterns were analyzed with GeneMapper software (Applied Biosystems) and compared to 26 reference strains from the European collection (Pr. Ed Kuijper, Leiden University) [7].

Definitions

Diarrhea was defined as three or more unformed stools per 24-h period. A CDI case was a patient with diarrhea and a positive stool test result (EIA for toxins A and/or B or positive toxigenic culture or immunochromatographic test or PCR) and/or endoscopically or histologically proven colitis due to *C. difficile*. Recurrence was defined if symptoms reappeared at least 10 days after, but no more than 2 months after, the resolution of the first episode, and was accompanied by a *C. difficile*-positive test result. All other cases of diarrhea were assumed non-CDI cases.

Outcomes examined

Outcomes of interest included the crude and attributable 30- and 60-day mortality rates and rates of complications. For each death, clinicians in charge of patients judged independently whether CDI was a primary cause, a contributory cause, or unrelated to the cause of death.

Statistical analysis

Outcomes in CDI-confirmed patients and those with diarrhea not related to *C. difficile* were described as follows: good (resolution of diarrhea) without complications, with complications, recurrences, in-hospital death, and death at D30 and D60. Rates of complications between CDI and non-CDI patients were also compared using chi-square or Fisher's exact test for qualitative variables in SPSS (version 17.0 for Windows, SPSS, Inc., Chicago, IL). A two-tailed *P* value < 0.05 was considered statistically significant in all tests.

Results

Between February 2011 and February 2014, 945 patients fulfilled the inclusion criteria and were included in the study: 233 with CDI and 712 with non-CDI diarrhea, which represented a CDI incidence of 2.15 per 1000 hospitalized patients or 3.11 per 10,000 hospital-days. The characteristics of study population are given in Table 1. There were more males than females: 52.5% vs 47.5%. The mean of length of stay at hospital was 31.1 days (min–max 2–414) with a median of 18 days (IQR 9–40.5). Forty percent of cases were diagnosed in the intensive care unit (ICU) or emergency room (patients hospitalized \geq 48 h in the emergency department).

Complications

Diarrheal episodes resolved without complication in 769 (81.4%) patients, and 87 (9.2%) died before resolution of their symptoms. The evolution of initial diarrheal episodes is described in Table 2. Overall, 105 (11.1%) patients developed at least one of the following complications (Table 3): colectomy, colitis, ileitis/rectitis, ileus, intestinal perforation, megacolon, multiorgan failure, pancolitis, peritonitis, PMC, renal failure, and sepsis/septic shock. Pancolitis, colitis, and acute renal failure were the most common complications. Complications occurred in 26.6% ($n = 62$) of patients with CDI and 6.0% ($n = 43$) with non-CDI diarrhea ($P < 0.001$). Among the 62 patients with complicated CDI, 45 (19.3%) developed one complication and 17 (7.3%) experienced at least two complications. The most frequent complications in the CDI group were colitis and/or pancolitis ($n = 33$, 14.2%), acute renal failure ($n = 24$, 10.3%), sepsis/septic shock ($n = 9$, 3.9%), ileitis/rectitis ($n = 5$, 2.2%), and colectomy ($n = 5$, 2.2%). Pseudomembranous colitis (PMC) was observed alone in three (1.3%) patients with CDI or associated with other complications in another four (1.7%) patients. Acute renal failure was diagnosed in 15 (88.2%) patients with more than one complication.

At D30, two patients had been admitted to ICU for CDI, six presented with renal failure, and two had colitis or septic shock. By D60, CDI was diagnosed in one patient with initial diarrhea not related to *C. difficile* and two patients were readmitted for diarrhea.

Mortality and recurrences

Overall, 848 and 808 patients were alive at D30 and D60, respectively, after the laboratory testing for CDI. At D60, the cumulative rate of recurrence was 13.7% ($n = 32$); 22 (9.4%) had recurrence of diarrhea by D30 and 10 (4.3%) from D30 to D60. By the end of follow-up, 137 (14.5%) patients had died. Among those with CDI, 25 (10.7%)

Table 1 Characteristics of patients included between February 2011 and February 2014

Variables	<i>N</i> = 945 patients (%)
Median age (IQR) (years)	68.1 (52.5–79.9)
Gender ratio (male:female)	1.1
Exposure to risk factors for CDI before onset of diarrhea	
In the last 60 days	
Antibiotics	595 (63.0)
In the last 30 days	
Antivirals	114 (12.1)
Immuno-suppressors	353 (37.4)
Medical procedures	346 (36.6)
Gastro-intestinal surgery	136 (14.4)
Nasogastric intubation	201 (21.3)
Stoma	41 (4.3)
Other	104 (11.0)
Recent hospitalization ¹	315 (33.3)
Residency in nursing home	43 (4.6)
Current use since at least 30 days	
Treatment with anti-acids	653 (69.1)
PPI	615 (65.1)
Anti H2	58 (6.1)
In the last 3 days	
Anti-diarrheal medication	40 (4.2)
Colic preparation	43 (4.6)
Laxatives	290 (30.7)
Underlying diseases	
Cardiovascular disease	562 (59.5)
Diabetes mellitus	261 (27.6)
Gastro-intestinal disease	171 (18.1)
Hepatic disease	111 (11.7)
Malignancy	250 (26.5)
Malnutrition (when patient was referred to dietitian)	333 (35.2)
Nervous system disease	177 (18.7)
Pulmonary disease	204 (21.6)
Renal disease	260 (27.5)
Systemic disease	50 (5.3)
Vascular peripheral disease	141 (14.9)
Other	275 (29.1)

¹ Before the first episode of diarrhea suspected to be related to *C. difficile*. CDI *C. difficile* infection, Anti H2 histamine H2 receptor blocker, PPI proton pump inhibitor. Variables were described by number and percentage (n (%)) except age described by median and interquartile (IQR)

died by D30 which increased to 37 (15.9%) total deaths within 60 days of diagnosis. Death was related to CDI in 15 patients (principal cause in 2 patients and contributing cause in 13 patients), which represented 40.5% (15/37) of deaths in those with CDI.

Table 2 Global evolution of diarrheal episodes in patients with CDI and in patients with diarrhea not related to *C. difficile*

Evolution of diarrheal episode	Study population, N = 945 (%)	Patients with confirmed CDI, N = 233 (%)	Patients with diarrhea not related to <i>C. difficile</i> , N = 712 (%)
Good, (cessation of diarrhea) without complications	769 (81.4)	155 (66.5)	614 (86.2)
Complications, then good	84 (8.9)	47 (20.2)	37 (5.2)
Good, (cessation of diarrhea) then death within 2 weeks	39 (4.1)	8 (3.4)	31 (4.4)
Death ¹	35 (3.7)	10 (4.3)	25 (3.5)
Complications, then death	13 (1.4)	8 (3.4)	5 (0.7)
Admission to ICU for CDI	5 (0.5)	5 (5.1)	0 (0)

¹ Diarrhea was present at date of death. *CDI C. difficile* infection. *ICU* intensive care unit. Variables were described by number and percentage (n (%))

Strains characterization

One hundred and twenty-three isolate (52.8%) from patients with CDI were available for typing. Eighteen PCR ribotypes were identified and 19 (15.4%) isolates had PCR ribotypes different from the 26 ribotypes used for comparison (Table 4). The six

main PCR ribotypes were 014/020/077 (23.6%; 29 isolates), 002 (12.2%; 15 isolates), 078/126 (8.9%; 11 isolates), 015 (6.5%; 8 isolates), 005 (4.9%; 6 isolates), and 001 (4.1%; 5 isolates), which accounted for 60.2% (74 isolates) of typed strains. Only one historical 027 strain (susceptible to erythromycin and moxifloxacin) was identified.

Table 3 Complications observed during the first episode of diarrhea

Number	Complications ¹ observed in patients during the first episode	CDI, N = 233 (%)	Not CDI, N = 712 (%)	P
1	Colectomy	0 (0)	2 (0.3)	1
	Colitis	14 (6.0)	8 (1.1)	< 10 ⁻³
	Ileitis/rectitis	4 (1.7)	3 (0.4)	0.12
	Ileus	0 (0)	1 (0.1)	1
	Pancolitis	13 (5.6)	3 (0.4)	< 10 ⁻³
	PMC	3 (1.9)	0 (0)	0.02
	Renal failure	9 (3.9)	20 (2.8)	0.56
	Sepsis/septic choc	2 (0.9)	1 (0.1)	0.56
2	Colitis + colectomy	1 (0.4)	0 (0)	0.56
	Colitis + septic shock	0 (0)	1 (0.1)	1
	Ileitis + pancolitis	0 (0)	2 (0.3)	1
	Ileitis + PMC	1 (0.4)	0 (0)	0.56
	Ileitis + renal failure	0 (0)	1 (0.1)	1
	Organ failure + renal failure	1 (0.4)	1 (0.1)	1
	Pancolitis + renal failure	2 (0.9)	0 (0)	0.10
	PMC + renal failure	2 (0.9)	0 (0)	0.10
	Peritonitis + renal failure	1 (0.4)	0 (0)	0.56
	Septic shock + renal failure	2 (0.9)	0 (0)	0.10
3	Renal failure + colectomy + septic shock	1 (0.4)	0 (0)	0.56
	Renal failure + colitis + ileus	1 (0.4)	0 (0)	0.56
	Renal failure + pancolitis + septic shock	1 (0.4)	0 (0)	0.56
4	Colitis + septic shock + pancolitis + renal failure	1 (0.4)	0 (0)	0.56
	Renal failure + peritonitis + colectomy + intestinal perforation	1 (0.4)	0 (0)	0.56
5	Organ failure + septic shock + renal failure + megacolon+ colectomy	1 (0.4)	0 (0)	0.56
6	Organ failure + septic shock + renal failure + PMC + ileus + colectomy	1 (0.4)	0 (0)	0.56

¹ Complications not including death. *CDI C. difficile* infection, *PMC* pseudomembranous colitis. Variables were described by number and percentage (n (%))

Table 4 Ribotypes in patients with CDI

PCR ribotypes	Typed strains <i>N</i> (%)	Development of complications during the first episode of CDI		Complications ¹ observed in CDI patients with typed strains (number)	Death by the end of follow-up <i>N</i> (%), <i>n</i> = 23	Death related to CDI (among typed strains) <i>N</i> (%)
		Non, <i>n</i> = 95	Yes, <i>n</i> = 29			
001	5 (4)	3 (3.2)	2 (6.9)	Colitis (1); pancolitis (1)	0 (0)	0 (0)
002	15 (12.1)	12 (12.6)	3 (10.3)	Colitis (2); PMC + renal failure (1)	7 (30.4)	1 (14.3)
003	4 (3.2)	3 (3.2)	1 (3.4)	Ileitis/rectitis (1)	1 (4.3)	1 (100)
005	6 (4.8)	5 (5.3)	1 (3.4)	PMC + renal failure (1)	1 (4.3)	0 (0)
012	3 (2.4)	2 (2.1)	1 (3.4)	Colitis (1)	0 (0)	0 (0)
014/020/077	29 (23.4)	27 (28.4)	2 (6.9)	Renal failure + colitis + ileus (1); renal failure + peritonitis + colectomy + intestinal perforation (1)	5 (21.7)	2 (40)
015	8 (6.5)	5 (5.3)	3 (10.3)	Renal failure (1)	0 (0)	0 (0)
019	2 (1.6)	0 (0)	2 (6.9)	Colitis (1)	0 (0)	0 (0)
023	2 (1.6)	1 (1.1)	1 (3.4)	Renal failure (1)	1 (4.3)	1 (100)
027	1 (0.8)	0 (0)	1 (3.4)	Sepsis (1)	1 (4.3)	1 (100)
029	2 (1.6)	2 (2.1)	0 (0)		0 (0)	0 (0)
046	1 (0.8)	1 (1.1)	0 (0)		0 (0)	0 (0)
050	3 (2.4)	3 (3.2)	0 (0)		0 (0)	0 (0)
056	1 (0.8)	1 (1.1)	0 (0)		0 (0)	0 (0)
078/126	11 (8.9)	8 (8.4)	3 (10.3)	Pancolitis (2); renal failure (1)	0 (0)	0 (0)
081	3 (2.4)	2 (2.1)	1 (3.4)	Colitis (1)	0 (0)	0 (0)
087	4 (3.2)	2 (2.1)	2 (6.9)	Pancolitis + renal failure (1); colitis + septic shock + pancolitis + renal failure (1)	1 (4.3)	1 (100)
106	4 (3.2)	3 (3.2)	2 (6.9)	Pancolitis (1); Organ failure + septic shock + renal failure + megacolon+ colectomy (1)	1 (4.3)	1 (100)
Others	19 (15.3)	15 (15.8)	4 (13.8)	Colitis (1); pancolitis (1); organ failure + renal failure (1)	5 (21.7)	1 (20)

¹ Complications not including death; *CDI C. difficile* infection; *PMC* pseudomembranous colitis. Variables were described by number and percentage (*n* (%))

Strains in two patients who developed recurrence were characterized as 078/126 and were similar in initial and recurrent episodes.

Twenty-three strains (62.2%; 23/37) were typed in patients with CDI who died and more than half of the isolates were of ribotypes 002 (30.4%) and 014/020/077 (21.7%). Among the deaths where CDI was listed as causative, five patients were infected with strains 003, 023, 027, 087, or 106.

Discussion

Over the past decade, the epidemiology of CDI has changed worldwide [3, 10–15]. The incidence of CDI in our hospitalized cohort was lower than in other studies, but comparable to a recent national study [7]. In 2013, the incidence increased as a consequence of increased sensitivity [16]. However, molecular techniques do not distinguish asymptomatic colonization from CDI; therefore, in our hospital, testing was limited to symptomatic patients.

In our prospective cohort study, the rate of complications was significantly higher in patients with confirmed CDI. Overall, 26.6% of patients with CDI experienced one or more complications, which is higher than the rate of 8 to 12.2% previously reported, even though definition of complicated CDI and duration of follow-up were not the same [17–20]. Most studies of patients with CDI have focused mainly on outcomes such as mortality, length of stay, recurrence, and cost [21, 22]. Although these are important, additional data on complications should be examined to evaluate the real burden of CDI.

Admissions to the ICU for CDI-associated complications and treatments for complicated disease such as colectomy are expected to rise [21]. The overall rate of colectomy in our study was 2.1% (5/233), slightly higher than report elsewhere (0.88–1.4%) [23–25]. Colectomy with megacolon, PMC, and ICU admission is frequently reported in the literature and commonly used to define the clinical presentation of CDI as severe/complicated or not severe/not complicated. This classification is important as it helps guide clinicians in the

management of infected patients, but not satisfactory for understanding the true impact of CDI. Many complications described in our study have not been adequately reported in terms of their frequency of occurrence in CDI, notably renal failure. Even the rate of CDI complications outside the large intestine remains to be fully determined.

Recurrence of diarrhea is particularly troublesome with CDI; there is 20–30% recurrence after an initial episode [21]. Such recurrent CDI diarrhea is often associated with prolonged hospital stays, excessive costs related to hospital readmission, and additional antibiotic therapy. The recurrence rate in our study (13.7%; 32/233) fits well with worldwide data (3.3 to 47.2%) despite the often different clinical definitions used in those earlier studies and used an arbitrary period (from first episode to recurrence), varying from 6 weeks to 1 year [20, 22, 26, 27]. A standardized duration of follow-up is required to define recurrence and ensure greater comparability between studies. Patients included in our study were followed actively for up to 60 days after diagnosis. We found that CDI recurrences in two cases were due to relapse with an antecedent strain of *C. difficile* which is consistent with other data [28].

Several studies have reported an increase in mortality since 2000 [29–31]. In our prospective study, CDI was considered to be the primary or a contributory cause in 15 (6.4%) deaths (up to D60), which appears a higher rate than reported for elsewhere in France (mortality at D30: 4% and at D60: 3.4%) and the Netherlands (3.3% at D30) [7, 25, 32]. However, this rate is similar to that reported elsewhere [33, 34]. In Europe, 30-day mortality varied between 6.8% in Ireland to 42% in the UK. The weighted average 30-day mortality ranged from 3% in France to 30% in the UK [22]. Heterogeneity among published studies in terms of definitions, patient groups, type and quality of study, duration of follow-up, and information collected does not allow the comparison of mortality rates in CDI patients [35].

The most common *C. difficile* ribotype identified in our study was similar to those reported recently in France [7]. The main PCR ribotypes identified in a recent multicentric European study were 027, 001/072, and 014/020 [36]. The distribution of PCR ribotypes may vary from one country to another and even from one region to another [7, 36]. PCR ribotype 027, although predominant in Europe, was identified in only one (0.8%) of the isolates typed in our study (the patient died with CDI listed as causative). Active surveillance with rapid action for new cases was ongoing during the study period, and no outbreaks were detected.

More than the half of the deaths in patients with CDI had ribotypes 002 and 014/020/077. Although no deaths in our study were attributed to these ribotypes, multiple studies have suggested that CDI may be more severe with certain ribotypes [37, 38]. However, a growing body of data does not provide support for the “hypervirulent” hypothesis, although the

clinical definition of outcomes or the methods used for data analysis were not consistent across all studies [39]. Indeed, the five deaths where CDI was attributed as causative in our study were with five different ribotypes. Overall, our findings indicate the little correlation between ribotype and severity of CDI.

The main strength of this study was that it was a prospective cohort study in which patients were actively followed for 2 months after CDI testing with no loss to follow-up. However, the study has some limitations. First, it was an observational study performed in a single university hospital. As such, caution is required in extrapolating our results to other healthcare facilities with different populations and approaches to managing CDI. Second, complications were recorded only when information was provided in patients’ medical records. As such, there is a possibility that some complications may be underestimated. Third, data concerning strain genotype characteristics was not available for all included patients, and it is also possible that the clonal diversity of *C. difficile* isolates was underestimated.

In summary, patients with CDI appear to have frequently more complications than those with diarrhea not related to *C. difficile*. To our knowledge, this is the first study that quantifies the unfavorable outcomes of CDI, providing practitioners with a good summary of the clinical profile of the burden in France. Vaccination would be an attractive preventative strategy in the management of symptomatic CDI in clinical practice.

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Compliance with ethical standards

Conflict of interest Nagham Khanafer received a PhD grant from Sanofi Pasteur, non-financial support (travel expenses) from Alere, Astellas, and Sanofi Pasteur, and personal fees from Astellas; Frédéric Barbut received personal fees and non-financial support (travel expenses) from Astellas Pharma Europe, Sanofi Pasteur, Pfizer, and Merck and scientific grants from Astellas Pharma Europe, bioMérieux, bioSynex, Cepheid, Cubist, Diasorin, Quidel-Buhlmann, and R-bioPharm; Philippe Vanhems received scientific grants from Sanofi Pasteur, Sanofi MSD, and Anios

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