Clostridium difficile infection: epidemiology, complications and recurrences

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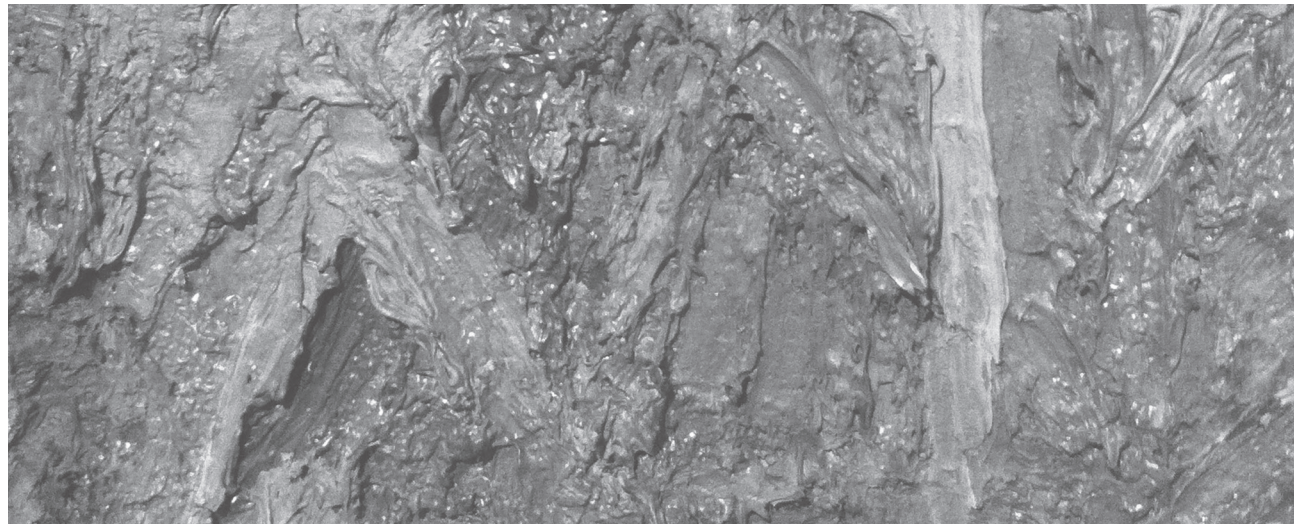

Background

Clostridium difficile is an anaerobic spore-forming bacillus that can be found in a wide range of habitats, from soil and water to intestines of animals, including humans. The bacterium was identified as the most important infectious cause of antibiotic-associated diarrhea in the 1970s [1]. *C. difficile* infection (CDI) is transmitted via the fecal-oral route. It has been associated mainly with hospitals, where it occurs both endemically and epidemically. However, since the beginning of the new millennium, the epidemiology of CDI appears to be changing. Higher incidence rates of CDI were recorded and large outbreaks with relatively high morbidity and mortality were noticed, first in Canada, followed by the US, the UK and the European mainland [2]. These outbreaks were found to be caused by a specific strain of *C. difficile*, typed as North American pulse field gel electrophoresis type I and PCR ribotype 027 [3]. This change in epidemiology renewed scientific interest in CDI, which led to more advanced understanding of the disease.

Pathogenesis

Much has been learnt about how *C. difficile* causes disease. This has been helped by molecular techniques, such as the construction of *C. difficile* mutants, and the availability of improved animal models. *C. difficile* spores, which are resistant to various physical and chemical attacks, may survive for years. Once they have been ingested and have passed the stomach, they germinate in the intestinal lumen under the influence of the binding of the primary bile acids cholic acid and cheodeoxycholic acid [4] to the receptor CspC [5]. The vegetative forms of the bacterium have to colonize the mucosa, a process that is greatly facilitated by disruption of the resident microbiome, usually as a result of antibiotics. The microbiome of CDI patients has less diversity than that of individuals without CDI. The proportion of lactate-producing bacteria is increased and that of butyrate-producing bacteria is decreased with great proportional losses of firmicutes and bacteriodetes [6]. A healthy microbiome may protect against colonization by *C. difficile* by metabolizing primary bile acids, competing for nutrients and mucosal surface, producing bacteriocins and influencing host defense [7]. The so-called surface layer proteins, especially SlpA, play an important role in adherence to the mucosal surface [8]. Pathogenic *C. difficile* strains produce the toxin TcdB and usually also TcdA. These large clostridial toxins probably leave the bacterial cell through a holin, coded by TcdE [9]. These toxins bind to unknown and probably different surface receptors on epithelial cells, and, after loss of epithelial barrier function, to underlying stromal cells. After binding, the toxins enter the cell through clathrin-mediated endocytosis [10]. Under the influence of decreasing pH in the endosome, TcdB undergoes a conformational change, resulting in its autocatalytic cleavage and release of the N-terminal catalytic domain in the cytoplasm

[11]. The autocatalytic activity of the toxins is partly countered by S-nitrosylation in the intoxicated cell [12]. In the cytoplasm, the toxins glycosylate Rho and Ras family GTPases [13, 14]. Glycosylation of RhoA, Rac1 and Cdc42 leads to loss of organization of the cytoskeleton, microscopically visible *in vitro* by characteristic rounding of intoxicated cells [15]. Although they have 48% homology, the toxins probably have different functions. TcdA appears to play a more important role in loss of epithelial cell polarity and epithelial integrity [16] and TcdB is more potent. TcdB-induced activation of Rac1 leads to assembly of the NADPH oxidase complex on endosomes, resulting in the formation of reactive oxygen species and eventually cell necrosis [17]. Both toxins appear to be capable of causing disease on their own [18]. The resulting pathologic effect is cell necrosis, fluid secretion and a massive influx of neutrophils, leading to the formation of cryptabscesses, which coalesce into macroscopically visible pseudomembranes (figure 1) [19]. Pathologic changes occur mainly in the colon, although ileitis has also been described, especially after colectomy [20]. Some strains also produce a third toxin, the binary toxin or *C. difficile* transferase (CDT), coded by the genes CdtA and CdtB. These genes are not part of the so-called Pathogenicity Locus, which contains genes for the large clostridial toxins. The lipolysis-stimulated lipoprotein receptor (LSR) has been identified as the receptor for CDT [21]. CDT modifies actin by binding ADP-ribose to it. Thus, it increases the cell surface available for bacterial adherence by induction of the formation of protrusions on the intoxicated cell [22]. Although this toxin is produced by the epidemic strain PCR ribotype 027, it is unclear how important this toxin is for its virulence. Certainly, *C. difficile* strains can cause severe disease without it.

Epidemiology

Admission to healthcare facilities and the use of antibiotics [23], which increase the risk of CDI for at least three months [24], are considered the most important risk factors for CDI. In addition, risk factors that have repeatedly been associated with the acquisition of CDI include advanced age [25], serious comorbidity [26], use of proton pump inhibitors [25, 27, 28], and failure to mount an antibody response against TcdA and TcdB [29, 30]. It is hard to pinpoint specific comorbidity predisposing to CDI and the severity of the comorbid illness seems more important than the exact nature. Proton pump inhibitor use is extremely difficult to separate from severe comorbidity, even after correction for confounding. Furthermore, a plausible biological mechanism by which proton pump inhibitors might predispose to CDI is lacking, since *C. difficile* spores, by which transmission mainly occurs, are acid-resistant. CDI has been regarded as a hospital-acquired infection, because patients admitted for other diseases develop CDI during their hospital stay and outbreaks have only been described in healthcare facilities. The hypothesis was that, even though *C. difficile* is a ubiquitous bacterium, it found a niche in hospitals and – to a lesser extent – nursing homes, where the most susceptible population of elderly, chronically ill individuals exposed to antibiotics is concentrated. This population might serve as a reservoir, in which *C. difficile* can multiply. The highly resilient spores are easily transmitted between patients, via the hands of hospital personnel, fomites [31] and even the air, which may contain spores in the vicinity of diarrheic patients [32, 33]. However, doubts have risen as to whether this model is entirely true. A large proportion of endemic hospital CDI cases cannot be linked to other cases in the same hospital [34] and community-onset cases with no apparent link to healthcare facilities have been described [35, 36]. Therefore, it seems likely that colonization with *C. difficile* often occurs outside of healthcare facilities and the disease only becomes symptomatic when other factors, such as the use of antibiotics, occur during a subsequent stay in a healthcare facility. If *C. difficile* colonization is acquired outside of healthcare facilities, what could be the reservoir? The meat industry has been implicated, since *C. difficile* has been cultured from meat products, albeit not consistently, and *C. difficile* is known to colonize and cause disease in farm animals, especially pigs [37]. Typing studies that found similarities between strains colonizing humans and animals have lent support to the hypothesis that animals can be a reservoir from which humans are colonized [38]. On the other hand, outbreaks with links to a food source or farm have not been described, and the link between humans and animals may also be explained by transmission from human to animal.

Various typing methods have been used for *C. difficile*, of which PCR ribotyping has gained greatest popularity. This method is based on the amplification of the variable-length spacer region between the two genes coding for the 16S and 23S ribosomal subunits [39]. Notable PCR ribotypes are the above-mentioned type 027, and type 078, which has been associated with farm animals [40]. Both of these PCR ribotypes are characterized by a deletion in TcdC, a putative negative regulator of toxin expression (although this function is debated [41, 42]), and production of CDT.

Clinical manifestations

The clinical manifestations of CDI vary. After ingestion of spores and successful colonization of the gut, asymptomatic carriage may follow, but in an estimated 15 to 30% colonized individuals [25, 43], symptomatic disease develops. This disease ranges from mild self-limiting diarrhea to fulminant colitis with a severe systemic inflammatory response, leukemoid reaction and ileus. The latter manifestation, which fortunately is rare, may lead to complications such as septic shock and perforation. This severe complicated form of CDI may be refractory to antimicrobial therapy. Usually though, CDI responds to antimicrobial therapy. In this case, the symptoms gradually improve over days to weeks. However, in some cases diarrhea relapses. The proportion of patients who suffer recurrences varies in studies from 6% to 77% depending on the number of previous CDI episodes [44-46], age [47-50], comorbidity

[50, 51], the need to continue antimicrobials for other infections than CDI [49, 50], humoral immune response [52-56], virulence of the *C. difficile* strain [57], treatment for CDI [58, 59] and, again arguably, the use of proton pump inhibitors [49, 60]. The majority of these recurrences are relapses, although some are reinfections [61, 62]. Without typing methods, which are not part of routine practice in most laboratories, this distinction is obviously difficult to make. An additional problem with this distinction is the fact that in some patients, more than one strain may be found at the same time [63]. The meaning of this is unclear as yet, although it seems most plausible that one of these strains represents the causative agent of the disease, whereas the others represent colonization. In order to distinguish between healthcare-associated and community-associated cases, and relapses and reinfections, arbitrary epidemiological criteria have been developed [64], although the biological ground for these criteria may be debated.

Diagnosis

The diagnosis of CDI is hampered by the fact that the distinction between colonization and disease is not always clear. Diagnostics can be based on the demonstration of free toxin (by cytotoxicity assay, based on demonstration of the above-mentioned characteristic cell rounding after exposure to patient feces *in vitro,* or by ELISA) or the bacterium in feces (by nucleic acid amplification test or culture) [65]. Demonstration of free toxin is thought to correlate better with disease as opposed to carriage [66], although toxin ELISAs are less sensitive than cytotoxicity assays. Diagnostic methods that demonstrate the bacterium instead of toxin may be better at distinguishing colonization from disease if they are quantitative [67].

Treatment

Mild CDI that develops during the use of antibiotics may be cured by stopping the antibiotic without directed treatment [68], but more-severe cases must be treated. As mentioned above, CDI usually responds to antibacterial therapy. Antibiotics that have traditionally been used are oral metronidazole and oral vancomycin (or related teicoplanin). The glycopeptides are generally considered slightly more effective than metronidazole on the basis of clinical studies and pharmacokinetics [69]. These antibiotics have the disadvantage that they cause collateral damage by harming the intestinal microbiome, thus predisposing to recurrences of CDI. Fidaxomicin, an antibiotic that came to market in 2011 for the treatment of CDI, has a narrower spectrum, and appears associated with fewer recurrences [58, 59]. Nevertheless, recurrences still do occur and remain the biggest challenge in treating CDI. Therefore, new antibiotics and other treatment modalities are still being searched for. Antimicrobials that are already available for treatment of other infections have been studied for the treatment of CDI. These include fusidic acid [70, 71], nitazoxanide [72, 73], rifaximin [74] and tigecyclin [75]. Several new compounds have been investigated for the treatment of CDI, of which the non-absorbable oxazolidinone cadazolid [76] and macrocyclic thiopeptide LFF571 [77] have been studied in published phase I clinical trials. The toxin-binding resin tolevamer was shown not to be effective [78]. Other treatment modalities may be divided in immunotherapy and microbial therapy. Immunotherapy concerns the administration of non-specific intravenous immunoglobulins or oral [79] or intravenous polyclonal or monoclonal antibodies directed against *C. difficile* and the large clostridial toxins, in order to supply additional antibodies when the patient fails to mount a sufficient humoral immune response. The intravenous administration of two monoclonal antibodies after antibiotic treatment for CDI resulted in a lower percentage of recurrences as compared to placebo [80]. However, selection bias may have been accountable for this result, because during the study, its endpoint was changed from reduction of symptoms in patients with diarrhea to reduction of recurrences in only those patients who became diarrhea-free. Microbial therapy concerns the administration of probiotics, donor feces or non-toxigenic *C. difficile* strains, in order to restore the microbiome and thus the colonization barrier against *C. difficile.* Of these, 'transplantation' of feces from healthy donors is currently the only therapy supported by a randomized trial [81]. There is no high-grade evidence on how to treat CDI when oral therapy is not possible, e.g., because of ileus. In severe cases of CDI with (imminent) toxic megacolon, surgery is the only remaining effective treatment. This consists of subtotal colectomy with end-ileostomy or, more recently, of the creation of a diverting loop ileostomy, followed by colonic lavage and flushing with vancomycin [82].

Prevention and control

Prevention and outbreak control measures are limited to prudent use of antibiotics (if necessary within the context of an antimicrobial stewardship program), adequate hand hygiene (with water and soap) and glove use, and disinfection of medical devices and surfaces of healthcare facilities with chlorine-containing solutions [83]. Isolation and cohorting of CDI patients seems a logical control measure, although there is no high-grade evidence for this. The role of asymptomatic carriers in the spread of *C. difficile* is unclear as yet. There is no convincing evidence that probiotics prevent CDI [84].

It is still unclear what the source of emerging *C. difficile* strains is and how they spread

The emergence of PCR ribotype 027 has been attributed to its acquisition of fluoroquinolone resistance and positive selection pressure from widespread use of these antimicrobials, and to increased virulence [85]. However, where the strain came from is unclear. In general, it is unclear what controls the dynamics of *C. difficile* strains. Where do new strains come from? How do they spread? What drives their spread? In particular, what is the role of the community and what is the role of asymptomatic carriers?

Better predictors of recurrence are needed to guide treatment of CDI

Many episodes of CDI will respond to stopping the inciting antibiotic or a first course of directed antimicrobial therapy. Some patients, however, will suffer one or multiple recurrences of CDI with associated protein-losing enteropathy, malnutrition, hypovolemia and even death. Identifying these patients may influence the choice of treatment. In such patients, an oral glycopeptide may be preferred over metronidazole, in spite of higher cost and (debatable) positive selection pressure for vancomycin-resistant enterococci [86]. In case of a really high risk of recurrence, it may be advantageous to choose costly fidaxomicin up front. A lower risk of recurrences as compared to vancomycin has been shown in patients with a first episode or first recurrence of CDI. Fidaxomicin has not been investigated in randomized trials in patients with multiple recurrences. Although it could be argued that treatment with a small-spectrum agent like fidaxomicin to prevent further loss of diversity of the microbiome in these patients is effective, it could also be argued that the advantage of the microbiome-sparing effect of fidaxomicin is lost in this patient category who have already lost most of the diversity of their microbiome. If the latter were true, it would be even more important to identify patients with a high risk of recurrence during their first episode. Predicting a high risk of recurrence might also lead to the decision to start adjunctive immunotherapy for CDI, or to administer donor feces. Unfortunately, predicting recurrence remains a major challenge, in spite of attempts to construct prediction scores [47].

Outline of this thesis

The research described in this thesis focuses on three issues united by a link to the major clinical challenge of CDI, the risk of a complicated or recurrent course: the distribution of *C. difficile* strains among various populations and recognition of strains associated with complications and/ or recurrences, predicting a complicated or recurrent course of CDI, and choosing therapy in order to minimize the risk of complications and recurrences.

Chapter 1 reviews what is known on community-acquired CDI, illustrated by two case reports of community-onset CDI.

In **chapter 2**, a study is reported that investigates community-onset cases of CDI in The Netherlands, focusing on risk factors and the distribution of *C. difficile* strains.

Chapter 3 reports a study into *C. difficile* carriage among patients with cystic fibrosis, a population in whom CDI is rare, despite the fact that they should be at high risk due to frequent contact with hospitals and high exposure to antibiotics.

Chapter 4 describes a study into the distribution of *C. difficile* strains among CDI cases across European hospitals and their clinical course.

In **chapter 5**, a case-control study is reported that investigated the value of one clinical marker and two biomarkers in predicting primary therapy failure and recurrence after initially successful therapy for CDI.

Chapter 6 describes a study into the association of antibody responses against large clostridial toxins and other *C. difficile* antigens with recurrence of CDI.

Chapter 7 describes a prospective interventional cohort study, in which participants received an experimental product made from whey of cows immunized with killed *C. difficile* and toxoid in addition to antimicrobial therapy for CDI, in order to reduce the risk of a recurrence.

Chapter 8 and **9** are the first version and an update of the guidance document issued by the European Society for Clinical Microbiology and Infectious Diseases for the treatment of CDI.

In the **summary and general discussion**, the conclusions of each chapter are summarized and suggestions for clinical practice and further research are made.

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Chapter 1

Community-onset *Clostridium difficile*-associated diarrhoea not associated with antibiotic usage. Two case reports with review of the changing epidemiology of *Clostridium difficile*-associated diarrhoea

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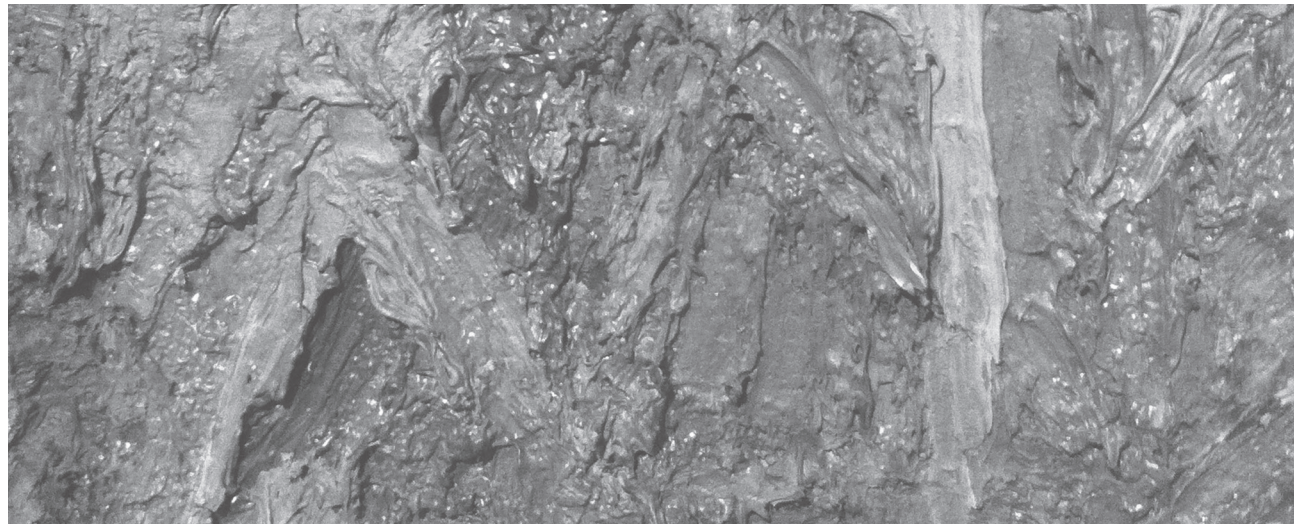

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Summary

The emergence of hypervirulent strains of *Clostridium difficile* causing outbreaks in hospitals and nursing homes may result in a greater than before spread of the bacterium in the community. By consequence the incidence of community-onset cases of *Clostridium difficile*–associated diarrhoea (CDAD) may increase outside known risk groups that are presently characterized by prior hospitalization, prior antibiotic usage, older age and significant co-morbidity. Herein, we describe two case histories of community-onset CDAD. The first concerns a previously healthy young female with community-acquired CDAD without recent hospitalization or antibiotic usage. The second patient developed diarrhoea in the community after discharge from a hospital where –in retrospect– an outbreak of CDAD occurred. The cases illustrate that CDAD should be included in the differential diagnosis of patients seeking care for community-onset diarrhoea, even in those without characteristic risk factors for CDAD.

Introduction

Recently, outbreaks of diarrhoea due to *Clostridium difficile* PCR-ribotype 027 have been reported in Canada, the United States and Europe, including The Netherlands.[1-5] Typically, outbreaks occur in a hospital or nursing home and primarily affect elderly individuals who suffer significant underlying conditions that make them susceptible to acquiring *C. difficile*-associated diarrhoea (CDAD).[6] Among predisposing conditions, the exposure to antibiotics during an extended period of hospitalization is regarded as most significant[7]. The severity of CDAD can range from transient, mild diarrhoea to fulminant colitis. A recently circulating strain of *C. difficile* characterized as toxinotype III, North American pulsed field type 1, restriction endonuclease analysis group BI and PCR ribotype 027 has been associated with enhanced virulence, apparently due to the production of higher amounts of toxins.[1,4,8]

As can be expected from the known risk factors of CDAD (i.e., hospitalization, old age, antibiotic usage, underlying medical conditions, gastro-intestinal surgery, nasogastric tubes, etc), most of the outbreak reports have dealt with nosocomial CDAD. There is an overall lack of information on community-onset CDAD. Herein, we describe two of such cases: a case of truly community-acquired CDAD without any known risk factors and a case of community-onset CDAD caused by an epidemic strain likely acquired during a recent stay in a hospital. The two cases illustrate various aspects of community-onset CDAD and indicate that physicians should be aware of the possibility of CDAD cases in the community, also in those who do not have known risk factors for CDAD. Moreover, such cases suggest that the epidemiology of CDAD may be changing, with a greater than before circulation of the bacterium in the community due to increased introduction of the bacterium from hospitals and institutions with outbreaks.

Case Report

A 28-year old female presented to the emergency department because of syncope and severe diarrhoea. Her prior medical history was unremarkable with the exception of two caesarean sections, performed years before the present admission. On presentation, she complained of cramping abdominal pain, nausea and vomiting of one day duration, and passing of profuse watery stools mixed with blood. Soon after her symptoms began she noticed light-headedness and briefly lost consciousness during passage of stools. Because of the peracute nature and severity of her symptoms, her general practitioner referred her to the emergency room of the nearby hospital.

The patient did not take any medication, nor had she recently used any. On physical examination, she did not appear severely ill. A blood pressure of 80/40

mmHg and a pulse of 60 beats per minute were noted. Examination of heart and lungs was unremarkable, her abdomen was tender. Rectal examination revealed pink stools with some mucus.

Laboratory investigation revealed a leukocytosis of 12.9 · 10^9/l, with a neutrophil count of 11.2 · 10^9/l. The haemoglobin level was 8.5 mmol/l and the ESR was 5 mm/h. Urea, creatinin, glucose, electrolyte and liver enzyme levels were within normal limits.

A diagnosis of vasovagal syncope due to a severe bout of gastro-enteritis and mild dehydration was made. The patient was admitted for fluid resuscitation (3 l in the first 24 h). Stool cultures were negative for *Salmonella, Shigella, Yersinia* and *Campylobacter* and stool examination did not reveal parasites like *Gardia lamblia*. An enzyme-linked immunosorbent assay (ELFA, Biomerieux) for *C. difficile* toxin A on stool was positive. Stool was not cultured for *C. difficile*. Treatment with oral metronidazole 500 mg tid for 10 days was initiated, a regimen to which she responded favourably. After 3 days she was discharged and completed her 10 day course of antibiotics at home. After completion of the antimicrobial regimen, she participated in an experimental protocol aimed at reducing the occurrence of relapses of CDAD and received a bovine immune milk preparation ('Anti-CD WPC') for two weeks. During a follow-up up to 60 days after start of the immune milk, the patient remained asymptomatic, and contact over one year later indicated that CDAD had not recurred.

Regarding the possible source of her *C. difficile* infection, an extensive history was taken. This revealed that three months ago the patient's two infant sons had been admitted to another hospital for two days because of a respiratory tract infection. The patient had spent one night in the hospital with her children during their admission. There had been no *C. difficile* outbreak in this hospital. Of note, neither the sons nor other family members had experienced diarrhoea. Moreover, after the diagnosis of CDAD had been made in the patient, stool cultures of her husband and two sons were taken but were negative for *C. difficile*. In conclusion, no plausible source of exposure could be established. It seems highly unlikely that the patient's episode of CDAD is related to her one day stay in hospital three months earlier.

The second case concerns a 71-year old male who was admitted with progressive diarrhoea. He had suffered a stroke in the past and had vascular dementia with secondary parkinsonism, chronic obstructive pulmonary disease, hypertension and chronic renal failure due to nephrosclerosis. One week before admission, he had been discharged from hospital where he was being examined for chronic watery stools with concomitant loss of an already compromised kidney function. Diarrhoea had been present for half a year. At that admission, there was peripheral eosinophilia (0.7 · 10^9/l). A CT-scan of the abdomen showed extensive arterial wall abnormalities compatible with atherosclerosis and thickening of the sigmoid wall. Colonoscopy had not revealed abnormalities, whereas biopsies of the sigmoid showed mild

inflammation with infiltration of eosinophils. Microbiological examination of stools including multiple tests for *C. difficile* toxin and extensive parasitological examination was negative. On the basis of these findings and especially the fact that repeatedly, no infectious agent could be demonstrated, a differential-diagnosis of eosinophilic colitis or cholesterol embolism was made. Symptomatic treatment with loperamide and haemodialysis was started. Upon readmission the patient had a fever up to 39.1 ^oC. His medication consisted of loperamide, aspirin, clopidogrel, atorvastatin, perindopril, metoprolol, temazepam, levodopa/carbidopa, alfacalcidol, epoetin beta iv during haemodialysis and ipratropium albuterol inhalations; he had not received antibiotics recently. The patient lived in a nursing home. Physical examination, including the abdomen, did not reveal abnormalities except for mild tachycardia of 110 beats per minute and increased bowel sounds. Laboratory investigation showed a leukocytosis of 15.6 · 10^9/l and an ESR of 48 mm/h. The eosinophilia had decreased. His C-reactive protein level was 322 mg/l. A rapid immunoassay for *C. difficile* toxin A (ELFA, BioMérieux) was positive. *C. difficile* was cultured from the stool as well; the strain was typed as ribotype 027. This case later on proved to be part of an epidemic due to ribotype 027 in this hospital. Treatment was started with oral vancomycin 250 mg qid for 14 days. After the antimicrobial regimen, the patient participated in the experimental protocol as well and received a bovine immune milk preparation ('Anti-CD WPC') for two weeks. His condition improved and he was discharged from the hospital; rapid stool tests for *C. difficile* toxins were repeatedly negative.

Discussion

The present cases concern community-onset CDAD. The first case illustrates that CDAD can be acquired in the community in the absence of any of the known risk factors for this disease. The second case illustrates how exposure to *C. difficile* in a hospital in which CDAD is endemic, can cause CDAD to spread into the community. It underlines once more that prior use of antibiotics is not a necessary factor for CDAD to develop.

Three factors are thought to explain the classical risk profile for CDAD. First, the patient must be exposed to the pathogen. Although the bacterium is ubiquitous and can be isolated from many sources both inside and outside hospitals, CDAD is most frequently acquired in hospitals and care institutions, where the bacterial load likely is high because host factors predispose the population admitted to these institutions to develop clinical disease.[6] Second, prior administration of antibiotics and consequent disruption of the resident bowel flora has always been considered important, if not necessary, for colonization by *C. difficile*. In particular clindamycin, cephalosporins, fluoroquinolones (especially of the later generations) and less so macrolides and

intravenous βlactams with βlactamase inhibitors have been associated with CDAD.[2,7,9-19] Lastly, a host factor appears to determine, at least in part, whether or not colonization is followed by clinical manifestations of CDAD. Older age cohorts admitted for extended periods because of severe underlying disease are at highest risk for CDAD.[7,19-22] Presumably, in these individuals a lack of effective anti-toxin humoral immunity is a decisive factor in developing CDAD, since long duration of disease and relapse has been associated with lower concentrations of circulating and faecal antibodies against *C. difficile* toxins A and B. A. [23-25]

Since early 2003, an increase in the incidence of CDAD has been reported in Canada and subsequently in the upper part of the United States of America and Europe. The CDAD cases in this outbreak were remarkable because they ran a more severe course.[19,26-28] The greater morbidity was associated with the emergence of PCR ribotype 027.[1,4,8] In just a few years, outbreaks of CDAD due to PCR ribotype 027 occurred in The Netherlands as well [3,4,29-31] Of note, the outbreaks concerned hospitalized or institutionalized patients. One report already noted an increase of community-acquired cases of CDAD in a population not considered at risk but unfortunately only a few strains of *C. difficile* were available for typing and type 027 was not found.[32]

The rate of community-acquired(CA-) CDAD, formerly a very rare entity, appears to be increasing.[19,33-35] Table 1 summarizes findings in the studies that have been published on this subject. Some of these cases may actually be hospital-acquired, since definitions of CA-CDAD vary. However, some clearly do not fit the classical risk profile.[19,32,36] A systematic surveillance of CA-CDAD has not been performed until recently. Stool samples of 703 patients with diarrhoea submitted by general practitioners in an area of 3.6 million inhabitants in Germany were investigated for pathogens including *C. difficile* by culture and enzyme immunoassay for *C. difficile* toxin A/B. The *C. difficile*-Toxin A/B assay was positive in 66 (9.3%) of the stool samples. Thirty-one (47%) of 66 patients had healthcare-associated diarrhoea (i.e., defined as an onset of symptoms within 4 weeks after hospital discharge) whereas 35 (53%) were truly community-acquired. Recent usage of antibiotics was reported by 34/66 (52%) patients, most frequently cephalosporins (33%) and fluoroquinolones (33%).[37]

If the incidence CA-CDAD is indeed increasing, what could be the cause? The emergence of CDAD in hospitals outbreaks undoubtedly leads to the spread of the pathogen among admitted patients, not all of whom will develop symptoms of CDAD during hospitalization. As illustrated by the second case, some cases of CDAD can be expected to occur in the weeks or even months following discharge. In addition, the increased circulation of *C. difficile* within hospitals will increase the rate of asymptomatic *C. difficile* carriership within the population, due to transient (?)

Table 1 Results of published studies concerning community-acquired CDAD.

Study	Country, year of study, study setting	Overall incidence of CDAD [/100,000 py]*	Number of patients with CDAD	Definition of CDAD	Proportion of CA-CDAD [%]	Definition of CA-CDAD	Proportion of COHA-CDAD [%]	Proportion of nosocomial CDAD [%]	Proportion of CDAD with unknown location of onset
Karlström[36]	Sweden, 1995, GP and hospital	58	1888	stool positive in any test for CD and CT+	28	CO, no hospitalization preceding 4 weeks	15	52	5
Kyne[33]	Ireland, 1995, hospital	?	73	diarrhoea and CT+	11	CO or onset in first 72 hours of admission without hospitalization preceding 60 days	9.6	79.4	0
Wheeler[34]	UK, 1993 to 1996, community	160	6	diarrhoea and CT+	100†	CO	?	0	0
Wheeler[34]	UK, 1993 to 1996, GP	20	17	diarrhoea and CT+	100†	CO	?	0	0
Dial[19]	UK, 1994 to 2004, GP	<1 in 1994 to 22 in 2004	1672	clinical diagnosis and/or CT+	74	CO without hospitalization preceding year	26	0	0
Paltansing[35]	Netherlands, 2005, hospital	16/ 10,000 admissions	81	diarrhoea and CT+	6§	CO	30§	61	3
Riley[49]	Australia, 1988 GP	5.5% of stool samples	16	diarrhoea, CD cultured or CT+	100†	CO	?	0	0

* unless otherwise specified
† CA-CDAD and COHA-CDAD were not separated
§ data partially through personal communication
Abbreviations: CDAD: *Clostridium difficile*-associated diarrhoea; py: person years; CA-CDAD: community-acquired CDAD; COHA-CDAD: community onset health care-associated; GP: general practice; CD: *Clostridium difficile*; CT+: cytotoxin test positive in stool; CO: community onset

excretion of the pathogen by discharged patients and or health care workers. Contact with such cases in the end will lead to some cases of community-acquired CDAD. Furthermore, it has been suggested that an animal reservoir may play a role in the emergence of community-acquired CDAD.[38] *C. difficile*-associated disease and carriage have been reported in pets and farm animals. In 1993, the role of pets as a reservoir was investigated comparing restriction endonuclease analysis types of *C. difficile* isolates from pets, veterinary clinics, humans and hospitals.[39] In that study, there was no correlation between isolates from pets and humans and therefore it was concluded that animals do not form an important reservoir for strains that cause human disease. However, *C. difficile* seems to become more important as an animal pathogen[40] and a number of recent studies have found overlap between animal and human ribotypes, suggesting that there is interchange of strains between animals and humans.[41,42] Of note, *C. difficile* could be cultured from 20% of retail meat samples in a Canadian study, with a majority of the toxigenic isolates being *C. difficile* type 027.[43] Incidentally, neither of the patients we describe had had contact with any possible animal source.

The first patient recovered quickly after treatment with metronidazole. In the recent outbreaks, however, the relapse rate of CDAD has increased from about 20% to as high as 47% in cases caused by PCR ribotype 027. Unfortunately, besides increasing the dose or extending the course of antibiotics, switching metronidazole into oral vancomycin and using alternating or pulsed regimens, there is little one can do to prevent cycles of relapses and even the measures mentioned are not proven efficacious. Also, the efficacy of strategies including probiotics, bacteriotherapy, toxin-absorbent resins and intravenous immunoglobulins is presently uncertain and not supported by evidence from clinical trials.[44-45] Previously, we reported on the use of passive immunotherapy with anti-*C. difficile* whey protein concentrate (40%; Anti-CD-WPC) made of milk from cows immunized with inactivated *C. difficile* toxins and killed bacterial cells. Anti-CD-WPC neutralizes the action of toxins in vitro and protects against CDAD in an animal model.[46] As a milk product, it was found safe for use in humans with CDAD[47] and in a first, uncontrolled trial an about 50 percent reduction in relapse rate was observed.[48] However, the efficacy of this treatment modality still has to be submitted to a dose finding and placebo-controlled randomized trial.

In conclusion, the emergence of new strains of *C. difficile* that cause outbreaks in hospitals and nursing homes in the last years may also forward the circulation of such strains in the general population, and increase the incidence of community-acquired cases of CDAD outside the well known risk groups. The present case histories illustrate that CDAD should be included in the differential diagnosis of both acute and

chronic community-onset diarrhoea, even when the patient has not recently taken antibiotics, is young and has no co-morbidity. It also underscores that strict hygienic measures should be taken in all patients with diarrhoea to prevent spread of the pathogen.

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Chapter 2

Clinical characteristics of community-onset *Clostridium difficile* infection in The Netherlands

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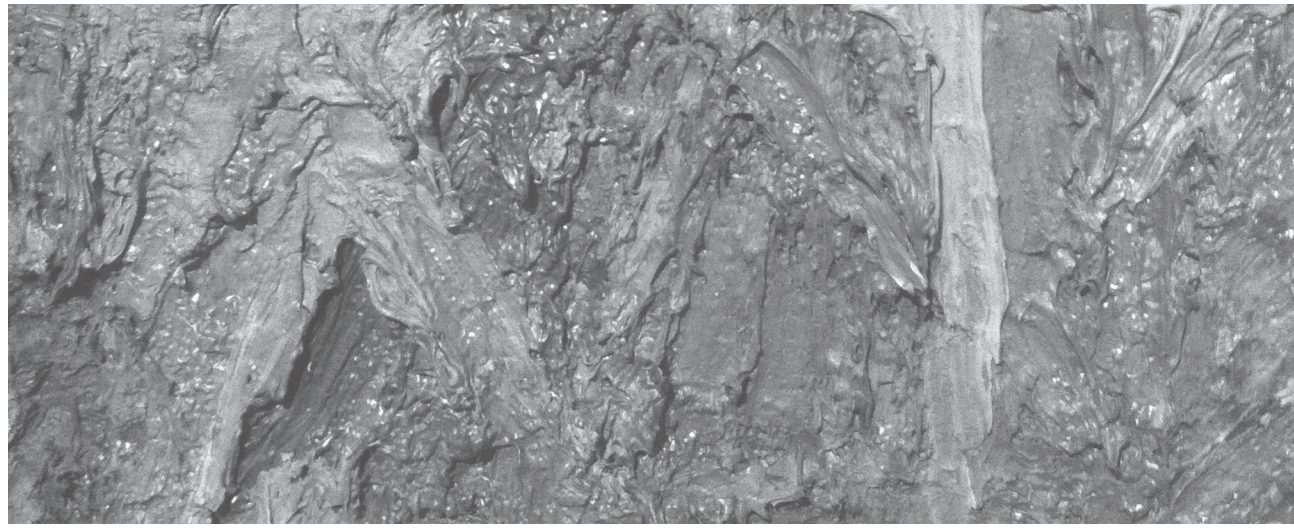

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Abstract

To elucidate the prevalence, characteristics and risk factors of community-onset Clostridium difficile infection (CO-CDI), an uncontrolled prospective study was performed. For 3 months in 2007–2008, three laboratories in The Netherlands tested all unformed stool samples submitted by general practitioners (GPs) for *C. difficile* by enzyme immunoassay for toxins A and B, irrespective of whether GPs specifically requested this. Patients with positive results were asked to complete a questionnaire. Positive stool samples were cultured for *C. difficile*, and isolates were characterized. In all, 2443 stool samples from 2423 patients were tested, and 37 patients (1.5%) with positive toxin test results were identified. Mixed infections were not found. Age varied from 1 to 92 years, and 18% were under the age of 20 years. Diarrhoea was typically frequent and watery, sometimes with admixture of blood or fever. Eight of 28 patients (29%) suffered recurrences. Among 31 patients with toxin-positive stool samples for whom information was available, 20 (65%) had not been admitted to a healthcare institution in the year before, 13 (42%) had not used antibiotics during the 6 months before, and eight (26%) had neither risk factor. A separate analysis for patients whose samples were both toxin-positive and culture-positive produced similar results. Cultured *C. difficile* isolates belonged to 13 different PCR ribotypes, and 24% of the isolates were non-typeable (rare or new) PCR ribotypes. In conclusion, CO-CDI can affect all age groups, and many patients do not have known risk factors. Several PCR ribotypes not encountered in hospital-associated outbreaks were found, suggesting the absence of a direct link between outbreaks and community-onset cases.

Introduction

Previously identified risk factors for Clostridium difficile infection (CDI) include admission to hospital or nursing home, old age, chronic comorbidity, longer hospital stay, antibiotic usage and prior chemotherapy [1], use of gastric acid sup- pressants, and nasogastric tubes. Since early 2003, both the incidence and the severity of cdi appear to have increased. This has been ascribed at least partly to the emergence of the new strain, PCR ribotype 027. In 2007, PCR ribotype 027 was found in stool samples of 25% of patients with nosocomial CDI in The Netherlands [2].

The incidence of cdi occurring outside healthcare facilities, usually termed community-onset CDI (CO-CDI), may be rising as well [3–10]. Some of the reported cases of CO-CDI may be truly community-acquired, but many cases may actually be linked to healthcare institutions. CO-CDI has never been investigated with detailed characterization of *C. difficile* isolates. In the present study, we aimed to investigate both the clinical characteristics and the source of CO-CDI in three areas in The Netherlands where outbreaks of nosocomial CDI due to PCR ribotype 027 had recently occurred. We screened all stool samples submitted by general practitioners (GPs) for *C. difficile*, characterized the cultured *C. difficile* isolates, and obtained patient information using a standardized questionnaire.

Materials and methods

Three medical microbiological laboratories in The Netherlands participated, namely SALTRO Artsenlaboratorium in Utrecht (providing services for 900 GPs), Public Health Laboratory Kennemerland in Haarlem (400 GPs) and the Laboratory for Medical Microbiology and Infectious Diseases in Zwolle (195 GPs). All unformed stool samples submitted by GPs during a period of three months were tested for *C. difficile*, using a commercially available rapid enzyme immunoassay (EIA) for *C. difficile* toxins A and B (ICTAB, Meridian). Samples were assayed irrespective of the diagnostic tests requested by the GP. If a stool sample tested positive and the corresponding patient had no earlier positive stool sample, this patient was included in our study.

Microbiological tests

If a stool sample gave positive results, the sample was cultured in the regional laboratory and isolates were sent to the reference laboratory at the Leiden University Medical Center. All isolates were genetically identified as *C. difficile* by an in-house PCR for the presence of the gluD gene specific for *C. difficile* [7]. *C. difficile* isolates were characterized by PCR ribotyping [11]. The presence of tcdA, tcdB, and binary toxin genes was investigated as described previously [2]. Antimicrobial susceptibility

was determined by E-test for erythromycin, clindamycin, moxifloxacin and ciprofloxacin, using the breakpoints recently described [12].

Clinical and epidemiological information

The laboratories collected demographic data from all patients whose stool samples were submitted by GPs. If a stool sample gave positive results, the GP who had submitted the sample was asked to give the patient an envelope containing information on the background and aim of our study, together with a request to complete a web-based or printed questionnaire.

The patients were asked for information concerning their symptoms, treatment and possible risk factors. The question about stool consistency on the day of maximal illness was illustrated by drawings from the Bristol Stool Scale [13]. We chose to enquire after antibiotic use during six months prior to diarrhoea instead of three months, because we wanted to rule out damage to the colonization barrier persisting longer than three months after the use of antibiotics. It is unclear how long this damage may persist, but, in animal models, persisting susceptibility to *C. difficile* colitis 74 days after one dose of clindamycin has been described [14]. If the patient could not or did not respond, we asked the GPs for the most essential patient information.

In June 2008, we asked the GPs of all included patients for information on persistent diarrhoea attributed to CDI, recurrences and deaths. The diagnosis of a recurrence was left to the judgment of the GPs.

Statistical analysis

Data were analysed with SPSS 14.0 for Windows (SPSS, Inc., Chicago, IL, USA). Descriptive statistics and Maentel-Haenszel-adjusted odds ratios were used to examine possible correlations. Non-normally distributed continuous variables were compared with the Mann-Whitney *U*-test.

Results

In total, 2443 stool samples from 2423 patients were submitted in by GPs. Thirty-seven (1.5%) patients with positive samples were identified. Of all 419 samples from patients aged 65 or older, 19 (4.5%) were toxin-positive. The laboratories in Utrecht and Zwolle registered whether GPs explicitly requested testing for *C. difficile*. This was the case in 12 of 32 positive stool samples.

Twenty-one patients completed a questionnaire. Information on ten of the remaining 16 patients was obtained from their GPs. We obtained follow-up information on 25 patients from their GPs in June, 2008.

Co-infection and characterization of the isolates

Co-infection of CDI with other enteropathogens was not found. Thirteen different PCR-ribotypes were found and seven strains could not be characterized by PCR-ribotyping (table 1). No *C. difficile* could be cultured from stool samples of five patients (14%) and the stools of three patients (8%) were not cultured due to logistical errors. As these eight patients may have had false positive stool toxin tests, we performed the analysis for all patients and for patients who had a positive culture. In spite of the fact that outbreaks due to the strain PCR-ribotype 027 had occurred in all regions, this PCR-ribotype was not found in the community.

Different PCR-ribotypes were not clearly linked to an age group or region, except for PCR-ribotype 078, which was found four times in the region of Zwolle and not in both other regions. The numbers of patients in each specific PCR-ribotype group were very small, limiting the possibility of finding associations with clinical characteristics.

Table 1 PCR-ribotypes found at various regional laboratories, followed by number (percentage) of isolates that contained binary toxin genes and number (percentage) that were resistant to various antibiotics.

PCR-ribotype	n isolates	binary toxin	moxiflo-xacin R	ciproflo-xacin R	erythro-mycin R	clinda-mycin R
002	1	0	0	1 (100)	1 (100)	0
014	3	0	0	3 (100)	1 (33)	1 (33)
015	2	0	0	2 (100)	0	0
023	1	1 (100)	0	1 (100)	0	0
025	1	0	0	1 (100)	1 (100)	0
043	1	0	0	1 (100)	0	0
044	1	0	0	1 (100)	1 (100)	0
067	1	0	0	1 (100)	0	0
078	4	3* (75)	0	4 (100)	2 (50)	1 (25)
081	1	0	1 (100)	1 (100)	0	0
110	1	0	0	1 (100)	1 (100)	0
117	2	0	1 (50)	2 (100)	0	0
172	3	0	0	3 (100)	0	0
unknown ribotype	7	0§†	0	6 (100) †	1 (17) †	0
no *C. difficile* was cultured	5					
no culture was performed	3					

* Two isolates contained only the gene for CdtA, not CdtB.
§ One isolate did not contain genes for TcdA or TcdB.
† One isolate was not available for binary toxin and susceptibility testing.

Five out of seven unknown PCR-ribotypes belonged to patients who had not been admitted to a hospital or nursing home and who were not employed in health care. A sixth patient had both been admitted to a hospital and a nursing home and for the seventh patient this information was not available.

The isolates were tested for antimicrobial susceptibility and production of binary toxin (table 1). Genes for binary toxin production were found in four isolates, which either belonged to PCR-ribotype 023 or 078, both of which have been associated with binary toxin production.

Clinical characteristics and follow-up information

Clinical patient characteristics are listed in table 2. Median ages of the patients with positive and with negative toxin stool tests were significantly different (54 years (range 1 – 92) and 37 (range 0 – 97), respectively; $p<0.001$). Symptoms were serious, with watery consistency, high stool frequency and often fever (27%) and admixture of blood (36%) and patients were usually treated (86%). The recurrence rate was high with eight (29%) of patients suffering recurrences and 1 patient (4%) still suffering from diarrhoea on follow-up. Of those eight patients who suffered a recurrence, six patients suffered one recurrence, one patient two recurrences and one four recurrences. Four out of 32 patients had died. Three deaths were deemed by the GPs not to be attributable to CDI and of one death this information was not available.

Risk factors

Risk factors we investigated are listed in table 3. Only 35% of patients had been admitted to health care facilities and only 58% had used antibiotics during the six months before diarrhoea developed. This percentage was similar among those who had been admitted (55%) and those who had not (60%). The antibiotics mentioned most often were amoxicillin/clavulanic acid (nine patients) and amoxicillin (4 other patients).

Most patients had comorbidity, as judged by the fact that 21 out of 31 (68%) used medication and 13 out of 21 (62%) reported being monitored by a medical specialist. When two patients who used only a selective serotonin reuptake inhibitor for a mood disorder and one patient who used only acetaminophen for pain because of osteoporosis were excluded, the percentage of patients who used medication fell to 58. Use of medication was associated with older age groups, but not restricted to these groups (lowest age quartile: none of six patients; both middle quartiles: two of nine patients; highest quartile: seven of seven patients). Gastric acid suppressants were used by 26% of patients. No patient was found to have a profession involving contact with farm animals.

Table 2 Clinical characteristics of CDI (sums of percentages may amount to more than 100 due to rounding)

	Toxin-positive		Toxin and culture-positive	
Characteristic (continuous variables)	**Median (range)**			
Age (years)	54	(1 – 92)	69	(1 – 92)
Interval between start of diarrhoea and stool test (days)*	10	(5 – 65)	12	(7 – 65)
Characteristic (categorical variables)	**Proportion (percentage)**			
Age category:				
0 – 4	2/ 37	(5)	1/ 29	(3)
5 – 9	1/ 37	(3)	1/ 29	(3)
10 – 14	2/ 37	(5)	2/ 29	(7)
15 – 19	2/ 37	(5)	2/ 29	(7)
20 – 39	4/ 37	(11)	2/ 29	(7)
40 – 59	7/ 37	(19)	4/ 29	(14)
60 - 79	8/ 37	(22)	8/ 29	(28)
≥ 80	10/ 37	(27)	9/ 29	(31)
Female sex	20/ 37	(54)	16/ 29	(55)
Stool consistency on the day of maximal illness:				
formed	1/ 21	(5)	1/ 16	(6)
mushy	1/ 21	(5)	1/ 16	(6)
watery	19/ 21	(90)	14/ 16	(88)
Stool frequency on the day of maximal illness (times per day):				
1 – 3	2/ 21	(10)	1/ 16	(6)
4 – 6	7/ 21	(33)	5/ 16	(31)
7 – 10	5/ 21	(24)	4/ 16	(25)
> 10	7/ 21	(33)	6/ 16	(38)
Admixture of blood with stools on any day	7/ 21	(33)	4/ 16	(25)
Abdominal pain on any day	14/ 21	(67)	9/ 16	(56)
Temperature over 38 °C on any day	5/ 21	(24)	3/ 16	(19)
Treatment:				
metronidazole	16/ 21	(76)	13/ 16	(81)
metronidazole, followed by vancomycin	2/ 21	(10)	2/ 16	(13)
no treatment	3/ 21	(14)	1/ 16	(6)
Course of diarrhoea:				
recovery from diarrhoea without antibiotics	9/ 28	(32)	7/ 22	(32)
recovery from diarrhoea after one treatment	10/ 28	(36)	8/ 22	(36)
recovery from treatment after ≥ 1 recurrences	8/ 28	(29)	7/ 22	(32)
persistent diarrhoea	1/28	(4)	0/ 22	(0)
Mortality:				
died	4/ 32	(13)	4/ 25	(16)
death partially attributable to CDI	0/ 31	(0)	0/ 24	(0)

* of the 21 patients who filled in the questionnaire

Table 3 Proportions (percentages) of patients with risk factors for CDI

Risk factor:	Toxin-positive		Toxin and culture-positive	
hospital admission in the year prior to diarrhoea and/or	9/ 31	(29)	8/ 25	(32)
admission to a nursing home in the year prior to diarrhoea	6/ 31	(19)	6/ 25	(24)
no admission to health care institutions in the year prior to diarrhoea	20/ 31	(65)	15/ 25	(60)
employment in health care	1/ 31	(3)	0/ 25	(0)
no admission to health care institutions in the year prior to diarrhoea or employment in health care	19/ 31	(61)	15/ 25	(60)
family members employed in health care	4/ 21	(19)	3/ 16	(19)
hospital admission of family members in the year prior to diarrhoea	2/ 21	(10)	2/ 16	(13)
visit to a nursing home in the year prior to diarrhoea	4/ 21	(19)	3/ 16	(19)
no link to healthcare institutions (as assessed by the above variables)	9/ 21	(43)	6/ 16	(38)
antibiotics during six months prior to diarrhoea	18/ 31	(58)	16/ 25	(64)
antibiotics during six months prior to diarrhoea of those not admitted to health care institutions in the year prior to diarrhoea	12/ 20	(60)	11/ 15	(73)
no admission to health care institutions in the year prior to diarrhoea or employment in health care or antibiotics during six months prior to diarrhoea	7/ 31	(23)	4/ 25	(16)
family members who experienced diarrhoea during the month prior to diarrhoea	5/ 19	(26)	4/ 14	(29)
use of medication	21/ 31	(68)	15/ 25	(60)
use of medication compatible with relevant comorbidity	18/ 31	(58)	14/ 25	(56)
use of corticosteroids	0/ 31	(0)	0/ 25	(0)
use of antiperistaltic agents	1/ 31	(3)	0/ 25	(0)
use of gastric acid suppressants	8/ 31	(26)	7/ 25	(28)
monitoring by a medical specialist (including nursing home physician)	13/ 21	(62)	12/ 16	(75)
pet ownership	6/ 21	(29)	4/ 16	(25)
professional contact with farm animals	0/ 31	(0)	0/ 25	(0)

Discussion

In this study of clinical and microbiological characteristics of CO-CDI, the prevalence of CDI among patients with community-onset diarrhoea for which microbiological diagnostics were requested amounted to 1.5%. In most cases, there was no specific request to test for *C. difficile*, which would have caused six out of ten cases to be missed. The clinical picture of the disease was severe with a high recurrence rate. We found no link to health care facilities in the majority of cases. Moreover, of the patients who were not admitted to healthcare institutions, 40% had not used antibiotics during the six months prior to the development of diarrhoea. Furthermore, 42% of all patients did not use medication compatible with relevant comorbidity and 18% were under 20 years of age. Finally, most of the PCR-ribotypes found were not associated with outbreaks in health care institutions. In particular, PCR-ribotype 027 was not found, in spite of the fact that in all of these areas outbreaks with this PCR-ribotype had recently occurred.

Methodological issues might have affected the results of this study. First, our study population was based on stool samples that were submitted by GPs, which may have led to referral bias. In The Netherlands, GPs are encouraged by their guidelines to culture stools when there is serious illness [15]. Therefore, it is possible that CO-CDI can run a much milder self-limiting course, in which no diagnostic tests are performed. Second, we screened for cases with an EIA for toxins A and B, and test characteristics will have influenced the population identified. EIAs may be relatively insensitive in comparison with stool culture and cytotoxicity assays [16]. We used an immunochromatography assay (ICTAB, Meridian), which has been shown to have a sensitivity of 91%, a specificity of 97%, a positive predictive value of 70% and a negative predictive value of 99% in comparison with the cytotoxicity assay used as the reference standard [17]. However, the characteristics of this assay were determined in a population of hospitalized patients and it is unknown if these characteristics may be extrapolated to a community setting. The design of the study was not optimized for a high recovery rate of *C. difficile* cultures, as each centre was allowed to apply its own culture protocol. This may have resulted in 14% toxin-positive and culture-negative stool samples. Alternatively, EIAs of these samples may have been falsely positive. Therefore, we analysed results from toxin-positive and culture-positive samples in a separate analysis. Third, bias may have been introduced by the manner in which clinical data were gathered. Part of the information came from questionnaires, which were completed by 21 of 37 patients. Possibly, the severity of diarrhoea or comorbidity of patients who completed the questionnaire differed from those who did not.

In spite of this possible bias, we feel that the strength of the study is the detail of the information that we did obtain. Most previous studies lack this detail, and no

other study has investigated the follow-up of patients with CO-CDI. Moreover, we characterized *C. difficile* isolates by ribotyping, which serves as an extra tool with which to investigate epidemiological associations.

Most studies on CO-CDI lack a clear definition of what is to be considered community-acquired. Often, CDI is designated as community-acquired when stool samples were collected in the community without knowledge of the patient's prior healthcare contacts. The European Centre for Disease Prevention and Control and the CDC have arbitrarily divided CO-CDI (and nosocomial CDI during the first 48 h of the admission) into community-onset healthcare facility-associated (CO-CDI occurring within 4 weeks after dis- charge from a healthcare facility) and community-acquired (occurring after 12 weeks after discharge) [18,19], leaving an intermediate period. Using these definitions, Kutty et al. [20] found many CO-CDI cases to be community-onset health- care facility-associated, suggesting that they were not actually acquired in the community, but in healthcare facilities. Only 17% of CO-CDI cases in a Dutch hospital-based surveillance study were community-acquired when the definitions of the European Centre for Disease Prevention and Control were applied [7]. The detail of the clinical information that we obtained allows for a clear distinction between CDI that is truly community-acquired and CDI that may have been acquired in healthcare facilities.

Furthermore, studies investigating CO-CDI seldom use molecular characterization of *C. difficile* isolates as an additional epidemiological tool. A Canadian study [21] characterized 17% of *C. difficile* strains from community sources as PCR ribotype 027, but no clinical data were available to verify that the patients had not been recently admitted to healthcare institutions.

A recent surveillance study by the CDC [10] found results very similar to ours. However, in this investigation, unlike ours, patients were not systematically surveyed and PCR ribotyping was not performed.

Finally, a recent case–control study in the UK [22] investigated the prevalence and clinical characteristics of patients with cytotoxin-positive stools submitted by GPs. The proportion of positive samples (2.1%) was consistent with that in our study. The proportions of patients who used antibiotics in the previous 4 weeks and who were hospitalized in the last 6 months were 52% and 45%, respectively. Unfortunately, no information was provided on comorbidity, animal contacts, follow-up and clinical characteristics of the diarrhoeal illness other than stool frequency. Also, the authors mentioned the frequent occurrence of PCR ribotype 001, but did not provide information on other PCR ribotypes found and whether these are associated with hospital outbreaks.

The incidence rate of CO-CDI cannot be estimated from our data, as it is unclear how many cases have been missed because patients did not visit their GPs or GPs did not perform diagnostic tests. Therefore, it is difficult to compare our findings with the results of surveillance studies of gastro- enteritis in Dutch general practices [23,24], which did not test for *C. difficile*.

Interestingly, our data suggest that CO-CDI does not directly result from the effects of healthcare-associated out- breaks. We did not find support for the hypothesis that an animal reservoir plays a major role in CO-CDI. However, in the region of Zwolle, PCR ribotype 078 was the most frequently encountered strain. This strain has frequently been found in recent surveillance studies of nosocomial CDI in The Netherlands. It has also been found in farm animals and meat products, and transmission from animals to humans seems possible. It was isolated from stools of diarrhoeal piglets in The Netherlands [25]. The city of Zwolle is situated in a rural part of The Netherlands, and one could speculate that a link between humans and animal cases exists in this area.

In conclusion, the prevalence of *C. difficile* in stools of patients with community-onset diarrhoea in The Netherlands for which diagnostics are requested by their GPs is 1.5%. All age groups can be affected, and many patients have not been admitted to healthcare institutions or used antibiotics. Many PCR ribotypes of *C. difficile* that are not encountered in hospital-associated outbreaks are found. Physicians, including GPs, should be aware of the possibility of CDI outside of the known risk factors.

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Transparency declaration

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Chapter 3

Patients with cystic fibrosis have a high carriage rate of non-toxigenic *Clostridium difficile*

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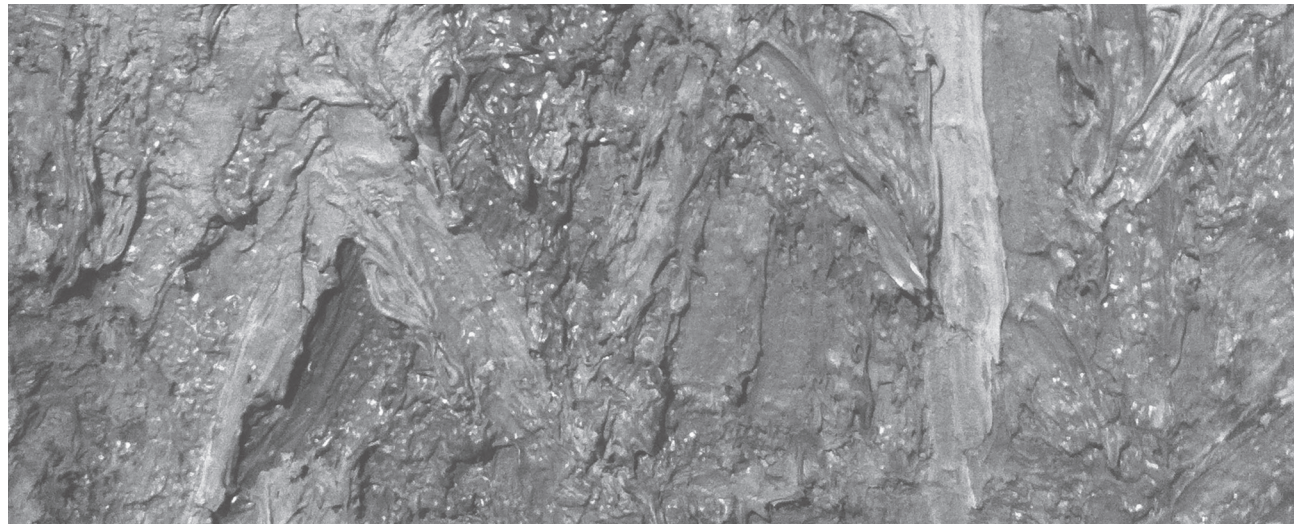

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Abstract

Thirty-year-old observations report frequent asymptomatic *Clostridium difficile* carriage among cystic fibrosis (CF) patients. In this case-control study, we found more carriers among CF patients than controls (47% versus 11%), but most strains carried by CF patients were non-toxigenic (77% versus 17%). Among CF patients, carriers were younger with more-severe pulmonary disease than non-carriers. Strains belonged to multiple PCR-ribotypes, suggesting that these CF patients did not acquire strains from each other.

Clostridium difficile infection (CDI) is an important cause of diarrhea and colitis. The most important risk factors are exposure to healthcare institutions and the use of antibiotics. Other associated factors are advanced age, severe comorbidity, decreased humoral immunity against *C. difficile* toxins and the use of proton pump inhibitors. Intestinal colonization with *C. difficile* may lead to disease, but also to asymptomatic carriage. The role of asymptomatic carriers in the spread of CDI is unclear as yet [1], as is the reason why some become carriers and others develop disease.

Several studies have suggested that patients with cystic fibrosis (CF) are often asymptomatic carriers of *C. difficile* [2-4], not surprising given their frequent use of antibiotics and exposure to hospitals. However, these observations were made in the 1980s, when the incidence of CDI was lower than now and epidemic strains such as PCR ribotype 027 had not yet emerged. Furthermore, the strains CF patients carry have not been characterized with molecular methods and predictors of *C. difficile* carriage among CF patients have not been investigated. Lastly, why CF patients apparently seldom develop disease remains unclear. Filling in these knowledge gaps may provide insight in CDI epidemiology and pathogenesis and have implications for infection prevention. In this case-control study, we sought to confirm earlier observations of frequent *C. difficile* carriage in CF patients, to characterize their *C. difficile* strains using molecular methods, to compare the aforementioned risk factors for CDI in this group with a control group and to identify predictors of *C. difficile* carriage in CF patients.

From June 2012 through November 2012, all adult CF patients monitored at Erasmus Medical Center, Rotterdam, a national CF center, were asked to participate in the study. The only exclusion criterion was failure to give informed consent. Inpatients submitted a stool sample on the ward, which was transported to Leiden University Medical Center the same day. Outpatients sent a stool sample by mail, which usually arrived the next day. Stool samples were cultured for *C. difficile* using selective media and the alcohol shock method [5]. Strains were characterized by PCR-ribotyping [6] and PCRs for toxin genes (TcdA, TcdB and binary toxin genes CdtA and CdtB) [5, 7, 8]. Patients described their bowel movements in a diary [9]. Clinical and epidemiological information was collected from patient charts.

As controls, we asked all patients present on 10 separate days between March 1st 2011 and September 30th 2011 on the internal medicine ward of Bronovo Hospital, The Hague, a general hospital with 815 beds, to participate in the study.

The distributions of continuous variables were compared using a Mann-Whitney *U* test. Pulmonary function test results were log-normalized for comparison and compared by *t* test. For associations between categorical variables, odds ratios (ORs) with 95% confidence intervals (95% CI) were calculated. IBM SPSS Statistics 20.0 software was used for the calculations.

Fifty-five CF patients and 108 controls submitted a stool sample. Twenty-six (47%) of CF patients were carriers versus 12 (11%) of controls (OR: 7.17; 95% CI: 3.22 – 16). Only 6 (23%) strains from CF patients were toxigenic, contrary to 10 (83%) strains from controls. Strains in both groups belonged to various PCR ribotypes (CF patients: 009, 010 (7 patients), 012 (2), 035, 039 (5), 046, 078, 097, 140, 151, 169, 207, unknown (3); controls: 018, 026, 043, 054, 076 (2), 081, 140, 142, unknown (2), 1 strain was not available). In only two strains, belonging to PCR ribotype 078 and an unknown ribotype, both from CF patients, genes for binary toxin were present.

None of 36 carriers had diarrhea (a mean of three watery bowel movements during three consecutive days), as opposed to five of 115 non-carriers; information was incomplete in 12 patients.

Among controls, the only statistically significant association was heart failure as defined in the Charlson comorbidity index [10] (Table 1).

CF patients had received more antibiotics than controls. Among CF patients, surprisingly, carriers were younger than non-carriers (Table 2). Also, carriers had worse pulmonary function parameters. Carriage was associated with severe (class I) mutations in the CFTR gene. Continuous variables were dichotomized using the median of the whole population as a cut-off. Because of an inverse relationship between age and pulmonary function, the odds ratios for pulmonary function parameters were adjusted for age by logistic regression analysis to correct for confounding. Age under 31 and FEV1 under 65% of predicted were significantly associated with carriage.

Strengths of this study were the detailed data, including daily defined antibiotic doses, pulmonary function tests and CFTR mutations, and the molecular characterization of *C. difficile* strains. A weakness of this study is the small number of patients. Furthermore, a comparison of CF patients with any other patient group is necessarily flawed because of differences in age and comorbidities. We tried to compensate for this by detailed documentation of risk factors for *C. difficile* carriage.

In the three studies from the 1980s, carriage rate among CF patients receiving antibiotics ranged from 22% to 50% versus 0% among those not receiving antibiotics [2-4]. Most of the colonized patients did not have diarrhea. Our finding of a high asymptomatic carriage rate is compatible with these studies, but only one study found a similarly low proportion of toxigenic strains. The association between carriage and more-severe pulmonary disease can probably be explained by higher antibiotic consumption. The association with younger age might tentatively be explained by a higher exposure to (non-toxigenic) strains circulating in the community.

Colonization with non-toxigenic *C. difficile* may protect against colonization with toxigenic strains [11] and may partially explain why CF patients seldom develop disease. Non-toxigenic strains might be less efficient at establishing long-term carriage than toxigenic *C. difficile* [12, 13]. We hypothesize that, due to differences in

colonic mucus or microbiome [14], non-toxigenic strains can colonize CF patients more efficiently than non-CF patients. The questions remain how non-toxigenic *C. difficile* strains can establish durable colonization in CF patients, and whether other factors than colonization by non-toxigenic strains protect CF patients from CDI.

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Transparency declaration

The authors declare no conflicts of interest.

Table 1 Characteristics of *C. difficile* carriers and non-carriers among controls.

	Carriage			No carriage			
Continuous variables	N	Median	IQR	N	Median	IQR	*P*
Age [years]	12	76	69 – 83	96	70	56 – 82	0.257
Total defined daily doses of antibiotics consumed in the previous 3 months	7	0	0 – 7	81	0	0 – 4	0.648
Charlson comorbidity index	12	3	0 – 4	96	2	0 – 3	0.531
Duration of current admission [days]	12	5	3 – 7	95	4	2 – 6	0.186

Categorical variables	N	%	N	%	OR	95%CI
Male sex	12	42	96	48	0.78	0.23 – 2.62
Use of antibiotics in the previous 3 months	12	67	96	44	2.57	0.73 – 9.12
Use of ceftazidime in the previous 3 months	12	8	96	1	8.64	0.51 - 148
Use of ciprofloxacine in the previous 3 months	12	33	93	15	2.82	0.75 – 11
Use of any immunosuppressant in the previous 3 months	12	17	95	19	0.86	0.17 – 4.25
Use of proton pump inhibitor in the previous 3 months	12	50	96	58	0.71	0.22 – 2.37
Hospital admission in the previous 3 months	12	33	96	20	2.03	0.55 – 7.44
Comorbidity as defined by Charlson index:						
History of myocardial infarction	12	25	96	15	1.95	0.47 – 8.11
Congestive heart failure	12	42	96	14	**4.56**	**1.26 – 17**
Peripheral vascular disease	12	8	96	5	1.66	0.18 – 15
Cerebrovascular disease	12	8	96	9	0.88	0.10 – 7.61
Dementia	12	0	96	2		
Chronic pulmonary disease	12	25	96	15	1.95	0.47 – 8.11
Rheumatologic disease	12	0	96	3		
Peptic ulcer disease	12	0	96	4		
Diabetes mellitus without microvascular disease	12	17	95	22	0.71	0.14 – 3.47
Diabetes mellitus with microvascular disease	12	8	96	4	2.09	0.21 – 20
Hemiplegia or paraplegia	12	0	95	1		
Renal disease	12	8	96	10	0.78	0.09 – 6.71
Non-hematologic malignancy	12	0	96	24		
Leukemia	12	8	96	0		
Lymphoma	12	8	96	2	4.27	0.36 – 51
Liver cirrhosis with portal hypertension or hepatic coma	12	17	96	0		
Metastasized non-hematologic malignancy	12	0	96	13		
HIV infection	12	0	96	0		

N = number of patients for whom information was available. IQR = interquartile range. OR = odds ratio. Results printed in boldface have reached statistical significance ($P < 0.05$).

Table 2 Characteristics of *C. difficile* carriers and non-carriers among cystic fibrosis patients

	Carriage			No carriage			
Continuous variables	N	Median	IQR	N	Median	IQR	*P*
Age	26	26	21 – 39	29	35	26 – 45	0.066
Total defined daily doses of antibiotics consumed in the previous 3 months	22	75	45 – 90	25	64	0 – 71	0.116
Forced expiratory flow between 25 to 75% of vital capacity [% of predicted]	23	16	9 – 52	28	29	12 – 58	0.227
Residual volume/ total lung capacity ratio [% of predicted]	21	115	84 – 146	28	91	80 – 114	0.054
Forced expiratory volume in 1 second [% of predicted]	23	54	32 – 80	29	73	52 – 89	0.085
Forced expiratory volume in 1 second/ vital capacity ratio [% of predicted]	23	65	60 – 80	29	74	59 – 93	0.378

Categorical variables	N	%	N	%	OR	95%CI
Age under 31	26	62	29	53	2.62	0.88 – 7.78
Age under 31, adjusted for forced expiratory volume in 1 second <65% of predicted					**3.98**	**1.08 – 14.67**
Forced expiratory flow between 25 to 75% of vital capacity <23% of predicted	23	57	28	43	1.73	0.57 – 5.28
Residual volume/ total lung capacity ratio ≥ 97% of predicted	21	67	28	46	2.31	0.71 – 7.45
Forced expiratory volume in 1 second <65% of predicted	23	65	29	38	3.07	0.98 – 9.59
Forced expiratory volume in 1 second <65% of predicted, adjusted for age less than 31					**4.61**	**1.25 – 17.03**
Forced expiratory volume in 1 second/ vital capacity ratio < 69% of predicted	26	54	29	38	1.91	0.65 – 5.60
Use of antibiotics in the previous 3 months	26	85	29	69	2.48	0.66 – 9.31
Use of ceftazidime in the previous 3 months	26	19	28	4	6.43	0.70 - 59
Use of azithromycin in the previous 3 months	26	73	29	48	2.91	0.94 – 9.02
Use of any immunosuppressant in the previous 3 months	23	26	29	14	2.21	0.54 – 9.01
Use of proton pump inhibitor in the previous 3 months	25	48	29	41	1.31	0.45 – 3.84
Admitted to hospital at the time of collection of stool sample	26	15	29	7	2.46	0.41 – 14.67
Hospital admission in the previous 3 months	26	12	29	7	1.76	0.27 – 11.5
Chronic sinusitis	25	52	28	32	2.29	0.75 – 6.98
Exocrine pancreatic insufficiency	26	85	29	72	2.10	0.55 – 8.01
Endocrine pancreatic insufficiency	26	27	29	28	0.97	0.29 – 3.18
History of meconium ileus or distal obstructive syndrome	26	12	29	10	1.13	0.21 – 6.16
Liver cirrhosis	26	8	29	10	0.72	0.11 – 4.70
Homozygous for ΔF508	22	50	29	48	1.07	0.35 – 3.25
Compound heterozygous for ΔF508	22	41	29	38	1.13	0.37 – 3.52
1 class I and 1 class II mutation	22	18	24	4	5.11	0.53 – 50
1 class I and 1 class III mutation	22	5	24	0		
1 class I and 1 class V mutation	22	5	24	0		
1 class I and second unknown mutation	22	0	25	8		
Any class 1 mutation	22	27	25	8	4.31	0.77 - 24
2 class II mutations	22	50	24	63	0.60	0.19 – 1.94
1 class II and 1 class IV mutation	22	0	24	4		
1 class II and 1 class V mutation	22	23	24	25	0.88	0.23 – 3.44
2 class IV mutations	22	0	24	4		

N = number of patients for whom information was available. IQR = interquartile range. OR = odds ratio. Results printed in boldface have reached statistical significance ($P < 0.05$).

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Chapter 4

Clostridium difficile infection in Europe: a hospital-based survey

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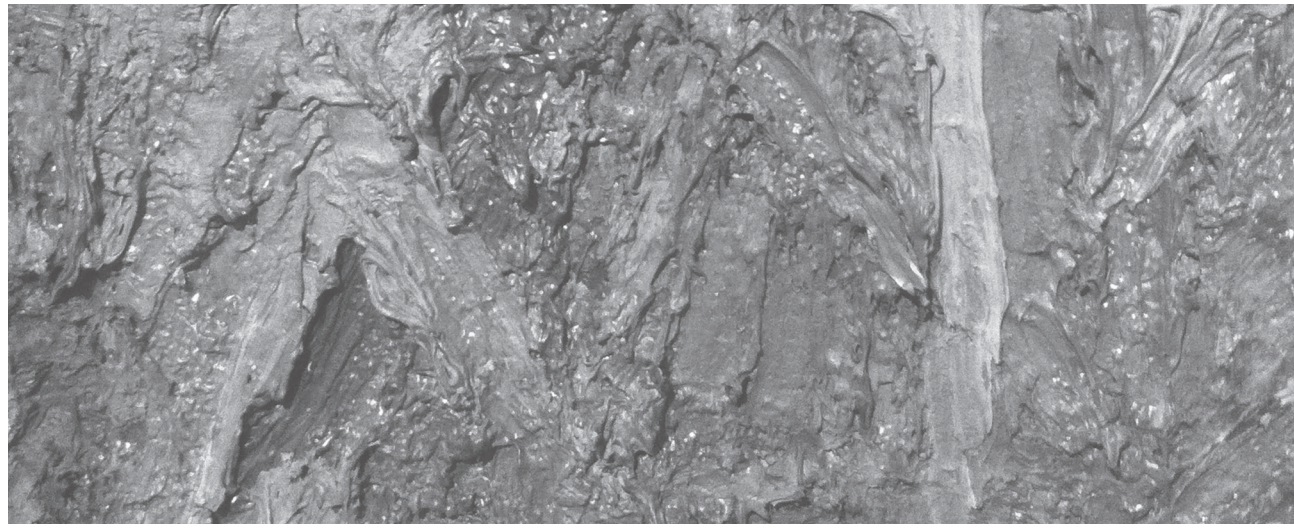

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Summary

Background

Little is known about the extent of *Clostridium difficile* infection in Europe. Our aim was to obtain a more complete overview of *C. difficile* infection in Europe and build capacity for diagnosis and surveillance.

Methods

We set up a network of 106 laboratories in 34 European countries. In November, 2008, one to six hospitals per country, relative to population size, tested stool samples of patients with suspected *C. difficile* infection or diarrhoea that developed 3 or more days after hospital admission. A case was defined when, subsequently, toxins were identified in stool samples. Detailed clinical data and stool isolates were collected for the first ten cases per hospital. After 3 months, clinical data were followed up.

Findings

The incidence of *C. difficile* infection varied across hospitals (weighted mean 4.1 per 10,000 patient-days per hospital, range 0.0-36.3). Detailed information was obtained for 509 patients. For 389 of these patients, isolates were available for characterisation. 65 different PCR ribotypes were identified, of which 014/020 (61 patients [16%]), 001 (37 [9%]), and 078 (31 [8%]) were the most prevalent. The prevalence of PCR-ribotype 027 was 5%. Most patients had a previously identified risk profile of old age, comorbidity, and recent antibiotic use. At follow up, 101 (22%) of 455 patients had died, and *C. difficile* infection played a part in 40 (40%) of deaths. After adjustment for potential confounders, an age of 65 years or older (adjusted odds ratio 3.26, 95% CI 1.08-9.78; p=0.026), and infection by PCR-ribotypes 018 (6.19, 1.28-29.81; p=0.023) and 056 (13.01; 1.14-148.26; p=0.039) were significantly associated with complicated disease outcome.

Interpretation

PCR ribotypes other than 027 are prevalent in European hospitals. The data emphasise the importance of multicountry surveillance to detect and control *C. difficile* infection in Europe.

Funding

European Centre for Disease Prevention and Control.

Introduction

Clostridium difficile infection is prevalent in health-care facilities throughout the developed world, but also presents as large outbreaks. Less often, it is acquired in the community from an unknown source. It characteristically occurs in elderly patients with comorbidity in whom the intestinal flora has been disrupted by previous use of antibiotics.[1,2] Since early 2003, increasing rates of *C. difficile* infection have been reported in Canada and the USA, with a larger proportion of severe and recurrent cases occurring in these countries than previously reported. The raised incidence and virulence of such infection have partly been explained by the spread of fluoroquinolone-resistant strains belonging to the PCR-ribotype 027.[3-5] In addition to the usual toxins A and B, these fluoroquinolone-resistant strains produce a binary toxin, with a hitherto uncertain pathogenic significance.[1-6] In Europe, PCR-ribotype 027 was first reported in 2005 in England and shortly thereafter in the Netherlands.[7,8] Subsequently, epidemics of *C. difficile* infection caused by PCR-ribotype 027 have been recognised in hospitals in many European countries.[9]

The attention given to this infection, diagnostic procedures in hospitals, presence and methodology of national surveillance, and availability of typing vary widely across Europe, which hampers comparisons between countries.[9,10] We did this study to obtain a more complete overview of the situation in Europe and build capacity for diagnosis and surveillance of *C. difficile* infection both nationally and Europe-wide.

Methods

Study design and patients

With support from the European Centre for Disease Prevention and Control, we appointed national coordinators for 34 European countries (including 27 member states, three candidate states, and four European-Free-Trade-Association countries) who selected hospitals in each country, relative to the country's population size. No randomisation was used for this selection. The aim was to include one hospital for countries with fewer than two million inhabitants, three for those with between two and 20 million inhabitants, and five for those with more than 20 million inhabitants, with a balance between academic and non-academic institutions. A study protocol noting all procedures was distributed to national coordinators and coordinators in all hospitals. Hospitals and laboratories completed a web-based questionnaire (Appendix) with epidemiological data, including numbers of patient-days, admissions, and stool samples tested for *C. difficile* infection in November, 2008, and technical data such as assays and culture methods used.

Procedures

Hospitals were asked to test for *C. difficile* infection in outpatients and inpatients suspected of having the infection by their treating physician and all inpatients who developed diarrhoea 3 days or more after admission. Clinical grounds on which to suspect recurrence were left to the attending physicians' judgment, who could use the definition of *C. difficile* infection according to the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) treatment guidance for *C. difficile* infection.[11] Only patients aged 2 years or older were included in the study. Patients with suspected *C. difficile* infection and diarrhoea, whose stool samples were positive for toxin A, B, or both (EIA, cytotoxicity test, or PCR) or revealed the presence of toxin-producing *C. difficile* were defined as having *C. difficile* infection.

A web-based questionnaire (Appendix) was used to gather additional information about demography, clinical data, and risk factors associated with the infection in the first patients to be diagnosed, with a maximum of ten patients included per participating hospital. If patients had episodes of *C. difficile* infection in the previous8 weeks, they were reported as having recurrent disease at inclusion. Stool samples from the first ten patients were cultured for *C. difficile* according to local protocols, and the isolates were sent to a central laboratory (Leiden University Medical Centre, Leiden, Netherlands) for further characterisation.

3 months after diagnosis, follow-up clinical data were obtained as part of the web-based questionnaire, including overall mortality, mortality attributable to *C. difficile* infection, colectomy, intensive-care-unit (ICU) admission, and recurrences during follow-up. Clinical grounds on which to suspect recurrence were left to the attending physicians' judgment, who could use the definition of recurrence according to the ESCMID treatment guidance for *C. difficile* infection.[11] All patients suspected of recurrence, who had toxin-positive-stool samples, were reported as having recurrence. No attempt was made to differentiate between relapses and reinfections. Identification of *C. difficile* was confirmed by an in-house PCR test for the glutamate dehydrogenase gene specific to *C. difficile*.[12] Isolates were further characterised by PCR ribotyping.[13] Since PCR-ribotypes 014 and 020 are nearly identical and differ only by one band on a specific agarose-gel electrophoresis, the types were reported together as ribotype 014/020. The presence of toxin A, toxin B, and binary toxin genes were investigated with standardised PCRs.[14,15] Isolates that were difficult to type were sent to the Anaerobe Reference Laboratory in Cardiff, UK, for further characterisation by the Cardiff PCR-ribotyping library, which currently consists of more than 300 ribotypes.[16] These isolates, and isolates of PCR ribotypes for which the toxinotype was unknown, were sent to the Institute of Public Health in Maribor, Slovenia, for toxinotyping.[17] No attempt was made to identify more than one causative ribotype, because infection by *C. difficile* resulting from more than one ribotype is thought to be rare.

We adhered to the epidemiological recommendations as defined by the ad hoc *C. difficile* surveillance working group.[1,18] Briefly, *C. difficile* infection is divided into health-care-associated cases (i.e., occurring in a hospital or nursing home after 48 h of admission or within 4 weeks after discharge from such a facility), community-associated cases (i.e., occurring in the community, provided that the patient had not been admitted to a health-care facility in the previous 12 weeks), and an indeterminate group for infections occurring between 4 and 12 weeks after discharge from a health-care facility. Furthermore, complicated disease was defined as *C. difficile* infection that contributed to or caused ICU admission or death, or led to colectomy. Severe comorbidity was defined as having a chronic-health points score over 0, as defined by the Acute Physiology, Age, Chronic Health Evaluation (APACHE) II score.[19] Quinolones were classified as old quinolones (nalidixic acid, norfloxacin, ofloxacin, ciprofloxacin) and new quinolones (levofloxacin, moxifloxacin, gatifloxacin).

Statistical analysis

For all hospitals, incidence rates of health-care-associated *C. difficile* infection were obtained by dividing the number of health-care-associated occurrences in November, 2008, (extrapolated by multiplication of the proportion of healthcare-associated infection in the questionnaires with all cases recorded in November, 2008) by the number of patient-days in November, 2008. Health-care-associated *C. difficile* infection incidence rates were also calculated with the total number of admissions as the denominator. Weighted mean incidence rates per hospital were calculated for each country from the incidence rates of all hospitals in that country, using the number of patient-days and the number of admissions per hospital as a weighting factor.

The associations of patient and pathogen characteristics with two outcome measures (complicated infections or recurrence within the 3-month follow up) were investigated. Since patients were nested within hospitals and might be exposed to common characteristics of their hospitals that could be important determinants of outcome, we could not assume independence of observations. Therefore, we chose a two-level multilevel-regression method, which takes into account within-group (hospital) and between-group relations, and allows for integration of hospital and patient variables. Since the outcome (complicated infection or recurrence) was binary, we used the logistic form of the multilevel-regression model. An odds ratio with a 95% CI was calculated for all associations between a patient or pathogen characteristic and an outcome—i.e., complicated infection or recurrence. Cases in which non-toxigenic strains were cultured were classified as culture negative, since these strains were not thought to be the cause of symptoms. Many of the associations reported in the analysis could be subject to confounding. For example, an association between the acquisition of *C. difficile* infection in a health-care facility (as opposed to

the community) and a complicated outcome might be confounded by age. To adjust the odds ratios for such potential confounders, we did a multivariate analysis for a selection of variables, again using a two-level logistic-regression model. As potential confounders, we selected variables for which a role as a confounder was biologically plausible and that were correlated to outcome with an alpha level less than 0.2, since significance-selection strategies to select for possible confounders do best at this level.[20] We tested whether confounders were highly collinear (variance inflation factor >10), in which case only one of them would be introduced as a covariate in multivariate analysis. Generally, statistical significance was declared for p values less than 0.05. Data were analysed with Stata 10.1.

Role of funding source

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Results

In total, 97 hospitals provided patients or epidemiological data, or both. Because some hospitals were unable to supply denominator data, we could not calculate incidences for all hospitals (table 1). Most hospitals were large, as judged by the number of patient-days and admissions (median number of admissions per month 2,645; IQR 1,808-4,257); 62 hospitals (67%) were academic hospitals. The estimated incidence of health-care-associated infection varied widely between hospitals. We calculated the proportion of health-care-associated *C. difficile* infection by the sum of health-care-associated and community-associated infections (table 1).

We tested associations between high-incidence hospitals (>10 per 10,000 patient-days) and antibiotics used by the patients in the month preceding inclusion. Use of aminopenicillins (odds ratio [OR] 2.70, 95% CI 1.17-6.22), first-generation cephalosporins (6.98, 1.83-26.62), or second-generation cephalosporins (2.40, 1.28-4.50) was significantly associated with high-incidence hospitals.

395 isolates from 73 hospitals in 26 countries were available for detailed characterisation. 65 different PCR ribotypes were identified (figure), including six new PCR ribotypes: 228, 229, 230, 231, 232, and 234. The most common PCR ribotypes were 014 and 020 (found in 19 countries), 001 (in 13 countries), and 078 (in 18 countries); PCR ribotype 027 ranked sixth (in six countries; table 2). Some commonly encountered PCR ribotypes were identified in a few countries and their distribution suggested regional spread (figure). Among these were PCR ribotype 106, which was reported in the UK (13 isolates), Ireland (five), and Spain (two), and PCR ribotype 018, which was recorded in Italy (19), Spain (two), Austria (one), and Slovenia (one). 12 different toxinotypes were identified. Of these, toxinotype 0 was most prevalent, representing 248 (65%) of 383 isolates; toxinotype III was identified predominantly in PCR-ribotype 027 strains (19 isolates) and only in five isolates belonging to rare PCR ribotypes (075, 099, 176, and 208); toxinotype IV predominantly in PCR-ribotype 023; and toxinotype V in PCR ribotypes 078 (30 isolates) and 126 (12); toxinotype XII fully correlated with PCR-ribotype 056. 13 (3%) isolates were *C. difficile*-toxin-A negative and *C. difficile*-toxin-B positive. 11 of these isolates belonged to PCR ribotype 017 and one each to the newly identified PCR ribotypes 232 and 234. Six (2%) isolates were non-toxigenic and were not included in further analyses.

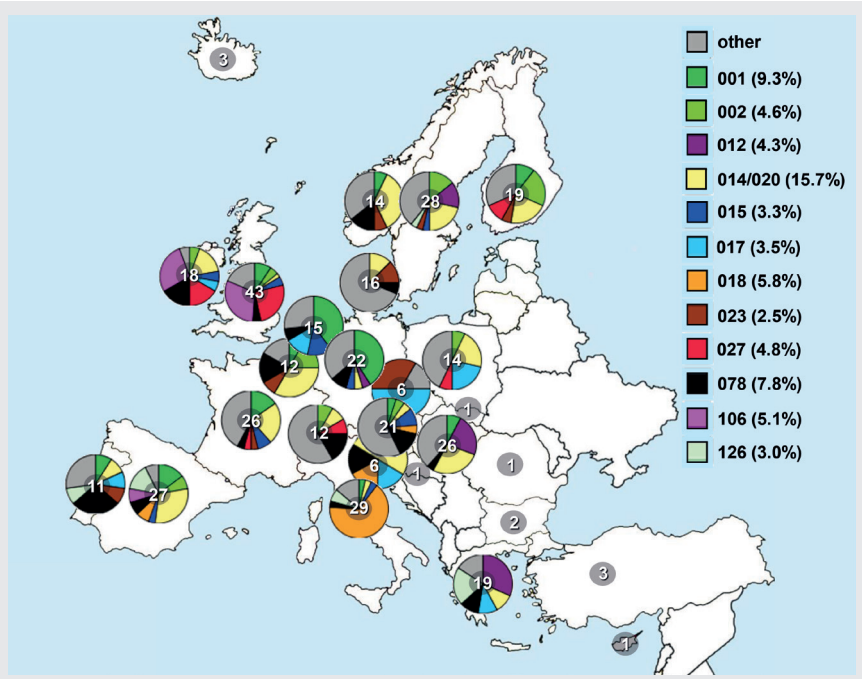

Figure Geographical distribution of *Clostridium difficile* PCR ribotypes in European countries with more than five typable isolates, November 2008.

Pie charts show proportion of most frequent PCR-ribotypes per country. The number in the centre of pie charts is the number of typed isolates in the country.

Table 1 Summary of *Clostridium difficile* infection in countries and hospitals.

	Number of toxin-positive cases/number of patients tested	Number of patients tested per 10,000 patient-days	Number of participating hospitals*	Weighted mean health-care-associated *C. difficile* infection incidence rate per hospital (minimum to maximum range) †		Percentage of health-care-associated *C. difficile* infection cases in health-care-associated and community-associated *C. difficile* infections	Number of complicated cases/ number of cases with available data (%)	Toxin tests used (number of hospitals)
				per 10,000 patient-days	per 10,000 admissions			
Austria	53/ 330 (16%)	52	3	7.5 (4.3 - 10.9)	36 (20 - 46)	92%	4/ 26 (15%)	A+B (2); A+B and Cu (1)
Belgium	16/ 283 (6%)	55	3	2.8 (0.0 - 6.2)	19 (0 - 39)	91%	0/ 11 (0%)	A+B (1); Cy and A+B (1); A (1)
Bulgaria	2/ 9 (22%)	3	3	0.6 (0.0 - 2.1)	3 (0 - 10)	100%	1/ 1 (100%)	A+B (3)
Croatia	22/ 197 (11%)	41	3 (2)	0.7 (0.5 - 2.1)	6 (4 - 20)	18%	1/ 14 (7%)	A+B (2)
Cyprus	1/ 28 (4%)	34	1	1.2	5	100%	0/ 1 (0%)	A+B (1)
Czech Republic	10/ 152 (7%)	17	3	1.1 (0.0 -1.3)	7 (0 - 9)	100%	2/ 7 (29%)	A+B (3)
Denmark	28/ 330 (8%)	74	3	5.5 (4.4 - 9.6)	18 (10 - 25)	88%	1/ 19 (5%)	A+B (1); Cu (2)
Finland	52/ 351 (15%)	141	3	19.1 (8.7 - 28.5)	80 (30 - 132)	91%	2/ 22 (9%)	A+B and Cu (1); Cu (1); A&B (1)
France	37/ 626 (6%)	42	5 (4)	2.1 (1.0 - 3.1)	15 (6 - 27)	84%	4/ 34 (12%)	A+B (2); Cu (1); Cy (1)
Germany	93/ 602 (15%)	72	6 (5)	7.4 (2.9 - 16.4)	60 (25 - 276)	91%	2/ 24 (8%)	A+B (3); Cu (1); Cy (1)
Greece	21/ 288 (9%)	60	3	3.7 (1.3 - 4.9)	29 (9 - 44)	84%	0/ 17 (0%)	A+B (3)
Hungary	22/ 333 (7%)	38	3	2.0 (0.4 - 3.9)	9 (1 - 23)	68%	1/ 25 (4%)	A+B (3)
Iceland	6/0	..	1	..	..	100%	0/ 6 (0%)	..
Ireland	38/ 493 (8%)	94	3	7.3 (6.5 - 7.9)	63 (39 - 92)	100%	5/ 21 (24%)	A+B (3)
Italy	57/ 533 (11%)	39	5	3.6 (0.4 - 5.8)	22 (2 - 61)	85%	5/ 18 (28%)	A+B (2), GluD and A+B (1); Cy (1)
Latvia	13/ 64 (20%)	10	3	1.9 (0.0 - 2.8)	13 (0 - 20)	91%	0/ 13 (0%)	A (2); A+B (1)
Luxembourg	0/ 28 (0%)	49	1	0.0	0	NA	0	A+B
Netherlands	18/ 309 (6%)	69	3	4.0 (2.3 - 8.5)	23 (13 - 43)	100%	1/ 15 (9%)	A+B (2); Cy (1)
Norway	37/ 241 (15%)	50	3	7.6 (0.4 - 16.5)	56 (3 - 229)	100%	1/ 16 (6%)	A+B (3)
Poland	102/ 263 (39%)	45	3	12.5 (3.8 - 36.3)	76 (29 - 189)	79%	1/ 11 (9%)	A+B (2); Cu (1)
Portugal	14/ 158 (9%)	45	3 (2)	2.6 (1.9 - 8.2)	13 (13 - 14)	86%	0/ 10 (0%)	A+B (3)
Romania	1/ 11 (9%)	3	5 (1)	0.3	2	100%	0/ 1 (0%)	A+B (2)
Slovakia	10/ 91 (11%)	16	3 (2)	1.4 (0.0 - 2.1)	11 (0 - 15)	71%	0/ 5 (0%)	A (1); Cu (1)
Slovenia	24/ 123 (20%)	17	3 (2)	2.8 (1.5 - 3.2)	19 (10 - 23)	67%	1/ 10 (10%)	A+B (2)
Spain	46/ 485 (9%)	45	5	4.3 (0.0 - 16.7)	30 (0 - 47)	100%	5/ 28 (18%)	A+B (2); Cu (1); A+B and Cy and Cu (1); A+B and Cu (1)
Sweden	69/ 430 (16%)	74	3	9.8 (6.3 - 15.7)	50 (28 - 71)	86%	2/ 30 (7%)	A+B (2); Cy (1)
Switzerland	16/ 150 (11%)	45	3	4.8 (0.0 - 7.5)	50 (0 - 84)	100%	0/ 12 (0%)	A+B (2); Cu (1)
Turkey	4/ 105 (4%)	4	5	0.0 (0.0 - 0.6)	0 (0 - 4)	20%	0/ 4 (0%)	A+B (3); A (1)
United Kingdom	164/ 1,695 (10%)	115	6	10.6 (6.7 - 30.3)	50 (44 - 135)	92%	5/ 40 (13%)	A+B (3); Cy (3)
Total	NA	NA	97 (87)	4.1 (0.0 - 36.3)	23 (0 - 276)	NA	44/ 442 (10%)	NA

A+B=enzyme immunoassay for *C. difficile* toxin A and B. A=enzyme immunoassay for *C. difficile* toxin A only. Cu=toxigenic culture. Cy=cytotoxicity test. GluD=enzyme immunoassay for *C. difficile*-specific glutamate dehydrogenase. NA=not applicable. ..=data not available. *Number of hospitals on which incidence data are based is shown in parentheses. The remaining hospitals did not provide denominator data. †Weight factor for weighted-mean incidence per 10,000 patient-days=number of patient-days; weight factor for weighted-mean incidence per 10,000 admissions=number of admissions. The UK and Germany were each granted one extra hospital. In Poland, three hospitals rather than five were recruited. No hospitals were recruited in Lithuania, and one was recruited in Malta. From Estonia, Liechtenstein, and the Former Yugoslav Republic of Macedonia no data or isolates were received.

Table 2 Characteristics of patients with *Clostridium difficile* infection for whom questionnaires were completed.

	n/ N (%)
Epidemiological characteristics	
Female	287/ 509 (56%)
Age ≥65 years*	319/ 509 (63%)
Epidemiological association	
Health-care associated	408/ 506 (80%)
Community associated	70/ 506 (14%)
Indeterminate association	28/ 506 (6%)
Explicit request to test for infection	441/ 507 (87%)
Use of an antibiotic not directed at *C. difficile* infection	
Any antibiotic not directed at *C. difficile* infection	366/ 463 (79%)
Aminopenicillin	28/ 463 (6%)
Aminopenicillin - β-lactamase inhibitor combination	86/ 463 (19%)
Antipseudomonal penicillin - β-lactamase inhibitor combination	38/ 463 (8%)
Second-generation cephalosporin	60/ 463 (13%)
Ceftazidime	78/ 463 (17%)
Any cephalosporin	155/ 463 (34%)
Carbapenem	41/ 463 (9%)
Aminoglycoside	27/ 463 (6%)
Old quinolone	80/ 463 (17%)
New quinolone	29/ 463 (6%)
Any quinolone	104/ 463 (23%)
Intravenous glycopeptide	33/ 463 (7%)
Lincosamide	28/ 463 (6%)
Macrolide	27/ 463 (6%)
Co-trimoxazole	25/ 463 (5%)
Use of any antibiotic not directed at *C. difficile* infection during previous 3 months	426/ 463 (92%)
Comorbidity	
Severe comorbidity (APACHE II CHP >0)	204/ 468 (44%)
Liver cirrhosis (APACHE II)	21/ 488 (4%)
Heart disease (APACHE II)	47/ 484 (10%)
Pulmonary disease (APACHE II)	54/ 480 (11%)
Chronic dialysis (APACHE II)	30/ 496 (6%)
Immunocompromised status (APACHE II)	106/ 488 (22%)
Treatment for inflammatory bowel disease	21/ 492 (4%)
Episodes of infection in previous 8 weeks	68/ 431 (16%)
Disease characteristics	
Outpatient	56/ 509 (11%)
Duration of diarrhoea	
<1 week	334/ 461 (73%)
1 to 3 weeks	92/ 461 (20%)
>3 weeks	35/ 461 (8%)

Table 2 Continued.

	n/ N (%)
Disease characteristics	
Diarrhoea mixed with blood at any moment in previous week	48/ 416 (12%)
Fever (temperature >38.5ºC)	167/ 446 (37%)
Ileus at any moment in previous week	20/ 509 (4%)
Last leukocyte count in previous week ≥15 × 10^9/L†	122/ 428 (29%)
Serum creatinine rise >50% compared to baseline before onset of symptoms	31/ 395 (8%)
Sigmoidoscopy or colonoscopy‡	
Pseudomembranes	7/ 29 (24%)
Ulceration	13/ 29 (45%)
Imaging‡	
Colonic wall thickening on CT	26/ 63 (41%)
Pericolonic fat stranding on CT	7/ 63 (11%)
Bowel distension on plain abdominal radiograph or CT	27/ 117 (23%)
Microbiological characteristics	
Most frequent PCR ribotypes among toxigenic isolates	
014/020	61/ 389 (16%)
001	37/ 389 (10%)
078	31/ 389 (8%)
018	23/ 389 (6%)
106	20/ 389 (5%)
027	19/ 389 (5%)
002	18/ 389 (5%)
012	17/ 389 (4%)
017	14/ 389 (4%)
015	13/ 389 (3%)
126	12/ 389 (3%)
023	10/ 389 (3%)
046	8/ 389 (2%)
003	7/ 389 (2%)
011	6/ 389 (2%)
053	6/ 389 (2%)
056	6/ 389 (2%)
Presence of either or both binary toxin genes in toxigenic isolates	90/ 389 (23%)
Toxin A negative, toxin B positive strains in toxigenic isolates	13/ 389 (3%)

All time periods mentioned are related to the time of collection of the stool sample. Only antibiotics that were administered to more than 5% of patients are given. APACHE II=acute physiology, age, chronic health evaluation version two. CHP=chronic health points. N=total number of patients for whom information was available.
*Median 71 (IQR 56 - 81). †Leucocyte count distribution ´10^9 per L (11; 11 - 15). ‡Data apply to current episode of *C.. difficile* infection. If several procedures were done during an episode, only the first was considered. §Two patients were treated for inflammatory bowel disease.

Most cases were health-care associated or community associated, leaving 6% of indeterminate association (table 2). Most patients fitted the previously established risk profile, with almost two-thirds aged 65 years or more, about two-fifths having severe comorbidity, and almost all having received antibiotics during the 3 months before their infection, most commonly cephalosporins, quinolones, and amino-penicillin - β-lactamase-inhibitor combinations (table 2). 68 (16%) of 431 patients had recurrent *C. difficile* at inclusion.

Data after 3-months' follow-up were obtained for about 90% of patients (table 3). An exact number cannot be provided, since follow-up was incomplete for some patients and therefore the number of patients with follow-up data differs for each variable. Of the 101 patients who had died, 40 (40%) of 101 deaths were judged to be related to *C. difficile* infection.

All seven patients who died from *C. difficile* infection as a main cause were aged 75 years or older and their infection was health-care associated. Six of them had severe comorbidity (four had pulmonary disease, three were immunocompromised, and two had heart disease). Two of these patients had a recurrent episode of infection at presentation. Two had leukocyte counts of 30 ´ 10^9 per L or greater and two of 4 ´ 10^9 per L or less. The strains causing these infections belonged to PCR-ribotypes 015, 018, 027 (two patients), and 056. No isolate could be obtained for two patients.

An age of 65 years or older, severe pulmonary comorbidity, previous use of a new quinolone, and infection by PCR-ribotypes 027, 015, and 018 were significant risk factors for complicated infections in univariate analysis (table 4). Patients with this comorbidity were distributed evenly among all hospitals. No disease characteristic—such as duration of diarrhoea, presence of fever, or leukocyte count—was significantly associated with complicated infection nor was the presence of binary toxin. After correction for potential confounders, an age of 65 years or older and infection by PCR-ribotypes 018 and 056 were significantly associated with complicated infection. These PCR ribotypes were binary-toxin negative and belonged to toxinotype 0 (type 018) and XII (type 056). The seven complicated cases caused by PCR-ribotype 018 occurred in four different hospitals in two countries, and the two complicated cases caused by PCR-ribotype 056 occurred in two hospitals in two countries.

An age of 65 years or older, previous use of ceftazidime, and recent episodes of *C. difficile* infection were significantly associated with recurrences during follow-up in univariate analysis (table 5). After correction for potential confounders, previous use of ceftazidime and recent episodes of infection were significantly associated with recurrence.

Since differences between patients with follow-up information and those without were possible, the characteristics of patients with available follow-up information about *C. difficile* infection complications (n=442) were compared with patients for whom this information was not available (n=67). Patients without this information were more likely to be outpatients at the time of presentation (OR 1.97, 95% CI 0.98 - 3.97), to have community-associated infection (2.59, 1.39 - 4.84), and be infected by PCR ribotype 018 (3.24, 1.20 - 8.73) or PCR ribotype 106 (3.96, 1.44 - 10.95); they were less likely to be aged 65 years or older (0.61, CI 0.36 - 1.02) and to have severe comorbidity (0.56, 0.31 - 1.01), especially pulmonary disease (0.26, 0.06 - 1.10). A separate analysis in which non-complicated *C. difficile* infection was assumed for patients with missing information resulted in closely similar values for the association of PCR-ribotype 018 with complicated infection (5.65; 1.63 - 19.57).

Table 3 Treatment and outcome (3-month follow up) characteristics of patients with *Clostridium difficile* infection.

	n/ N (%)
Initial episode treated with	
Oral metronidazole	341/ 477 (71%)
Intravenous metronidazole	50/ 472 (11%)
Oral vancomycin	89/ 483 (18%)
Intracolonic vancomycin	1/ 473 (0.2%)
ICU admissions	31/ 459 (7%)
CDI contributive	6/ 459 (1%)
CDI primary cause	1/ 459 (0.2%)
Colectomy for CDI	3/ 460 (0.7%)
Death	101/ 455 (22%)
CDI contributive	33/ 455 (7%)
CDI primary cause	7/ 455 (2%)
Complicated CDI	44/ 442 (10%)
Recurrent CDI*	86/ 484 (18%)
Both complicated and recurrent CDI	10/ 440 (2%)

Of 491 (96%) of 509 patients, complete or partial follow-up information was available. n=characteristics of patients with *Clostridium difficile* for whom questionnaires were completed. N=total number studied. ICU=intensive care unit. CDI=*C. difficile* infection. *Number of recurrences during follow-up in those patients who had recurrences: median 1; 1 - 3.

Because death or colectomy could have precluded a patient from having a recurrence, a separate analysis was done for risk factors for recurrence in only those patients who did not die or undergo a colectomy. Results of the univariate analysis mirrored the analysis for the whole group, except that previous use of intravenous glycopeptides and chronic dialysis were significantly associated with recurrence (3.28, 1.12 - 13.78 and 2.87, 1.02 - 8.14, respectively).

Different cutoff values for the continuous variables age and leukocyte count, as assessed by receiver operator characteristics, did not lead to improved performance in the prediction of complicated *C. difficile* infection.

Table 4 Determinants of complicated *Clostridium difficile* infection

	Univariate analysis			Multivariate analysis		
	OR	95% CI	p	OR	95% CI	p
Epidemiological characteristics						
Age ≥65 years	4.84	1.78 - 13.13	0.002	3.26*	1.08 - 9.78	0.035
Health-care-associated *vs.* community-associated and indeterminate infection	3.23	0.92 - 11.40	0.068	4.86*	0.59 - 40.04	0.141
Severe comorbidity (APACHE II CHP >0)	1.17	0.57 - 2.40	0.666	..	..	..
Liver cirrhosis (APACHE II)	0.53	0.06 - 4.56	0.562	..	..	..
Heart disease (APACHE II)	1.71	0.62 - 4.76	0.302	..	..	..
Pulmonary disease (APACHE II)	2.66	1.11 - 6.37	0.028	1.38*	0.48 - 4.02	0.543
Chronic dialysis (APACHE II)	0.29	0.04 - 2.35	0.248	..	..	..
Immunocompromised status (APACHE II)	0.92	0.39 - 2.17	0.850	..	..	..
Treatment for inflammatory bowel disease†	..	..	..	..	..	..
Use of an antibiotic not directed at *C. difficile* infection during previous month						
Aminopenicillin	2.69	0.69 - 10.51	0.156	2.39*	0.43 - 13.33	0.320
Aminopenicillin - β-lactamase inhibitor combination	1.81	0.80 - 4.06	0.153	1.18*	0.43 - 3.23	0.741
Antipseudomonal penicillin - β-lactamase inhibitor combination‡	..	..	..	..	..	..
Second-generation cephalosporin	0.53	0.14 - 1.97	0.343	..	..	..
Ceftazidime	1.34	0.52 - 3.46	0.546	..	..	..
Any cephalosporin	0.92	0.42 - 2.02	0.831	..	..	..
Carbapenem	1.29	0.42 - 4.00	0.657	..	..	..
Aminoglycoside	1.65	0.45 - 6.05	0.453	..	..	..
Old quinolone	1.41	0.57 - 3.53	0.459	..	..	..
New quinolone	3.45	1.07 - 11.06	0.038	2.57*	0.68 - 9.72	0.163
Any quinolone	2.29	1.03 - 5.09	0.043	..	..	..
Intravenous glycopeptide	1.95	0.61 - 6.20	0.257	..	..	..
Lincosamide	0.32	0.04 - 2. 79	0.303	..	..	..
Macrolide	2.69	0.80 - 9.00	0.108	4.60*	0.72 - 29.37	0.107
Co-trimoxazole	0.33	0.04 - 2.83	0.321	..	..	..
Episodes of infectionl in 8 weeks before current episode	0.77	0.27 - 2.19	0.621	..	..	..
***Clostridium difficile* infection characteristics**						
Duration of diarrhoea >1 week	0.55	0.23 - 1.32	0.182	..	..	..
Diarrhoea mixed with blood	1.06	0.33 - 3.42	0.928	..	..	..
Fever (temperature >38.5°C)	1.28	0.59 - 2.76	0.533	..	..	..
Ileus	2.84	0.73 - 11.08	0.132	..	..	..
Leukocyte count ≥15 × 10^9/L	1.50	0.67 - 3.35	0.324	..	..	..
Serum creatinine rise >50%	2.33	0.63 - 8.63	0.205	..	..	..
Bowel distension	2.06	0.38 - 11.25	0.405	..	..	..
Microbiological characteristics						
PCR-ribotype 027§	4.72	1.34 - 16.56	0.016	2.56¶	0.64 - 10.25	0.184
PCR-ribotype 078§	1.08	0.29 - 4.10	0.909	..	..	..
PCR-ribotype 014/020§	0.43	0.12 - 1.50	0.184	0.60¶	0.17 - 2.16	0.433
PCR-ribotype 015§	3.77	1.01 - 14.08	0.048	4.56¶	0.98 - 21.20	0.053
PCR-ribotype 018§	9.22	2.24 - 38.09	0.002	6.19¶	1.28 - 29.81	0.023
PCR-ribotype 023§	1.00	0.11 - 9.11	0.999	..	..	..
PCR-ribotype 056§	10.96	0.96 - 126	0.054	13.01¶	1.14 - 148.26	0.039
Presence of either or both binary toxin genes	1.09	0.46 - 2.54	0.847	..	..	..
Toxin A negative, toxin B positive strains *vs.* all other strains	0.69	0.08 - 6.08	0.739	..	..	..
Toxinotype III (including IIIb and IIIc) *vs.* all other toxinotypes	3.18	0.96 - 10.56	0.059	1.81¶	0.48 - 6.75	0.378

OR=odds ratio. APACHE II=acute physiology, age, chronic health evaluation version II. CHP=chronic health points. ..=data not available. *Adjusted for other variables: age ≥65 years, health-care association, pulmonary disease, previous use of aminopenicillin, previous use of aminopenicillin with β-lactamase inhibitor, previous use of a new quinolone, previous use of macrolide, PCR-ribotype 027, PCR-ribotype 014/020, and PCR ribotype 056. †No complicated *Clostridium diff cile* infection occurred in 16 patients treated for inflammatory bowel disease *versus* 44 cases of complicated *C. difficile* infection in 419 patients without inflammatory bowel disease. ‡No cases of complicated *C. difficile* infection occurred in 34 patients who received an antipseudomonal penicillin–β-lactamase inhibitor combination *versus* 43 cases of complicated *C. difficile* infection in 381 patients who did not receive drug combination. §*Versus* all other ribotypes. ¶Adjusted for other variables: age ≥65 years, health-care association, pulmonary disease, previous use of aminopenicillin, previous use of aminopenicillin with β-lactamase inhibitor, previous use of a new quinolone, previous use of macrolide.

Table 5 Determinants of recurrence of *Clostridium difficile* infection during follow-up

	Univariate			Multivariate		
	OR	95%CI	p	OR	95%CI	p
Epidemiological characteristics						
Age ≥65 years	1.91	1.08 - 3.37	0.026	1.86*	0.88 - 3.92	0.104
Health-care-associated *versus* community-associated and indeterminate	1.77	0.83 - 3.78	0.139	1.93*	0.59 - 6.35	0.278
Severe comorbidity (APACHE II CHP >0)	1.35	0.79 - 2.31	0.273	..	..	..
Liver cirrhosis (APACHE II)	0.50	0.11 - 2.33	0.375	..	..	..
Heart disease (APACHE II)	1.16	0.50 - 2.68	0.734	..	..	..
Pulmonary disease (APACHE II)	0.51	0.20 - 1.32	0.165	0.62*	0.20 - 1.95	0.417
Chronic dialysis (APACHE II)	2.04	0.79 - 5.26	0.139	2.23*	0.59 - 8.37	0.235
Immunocompromised status (APACHE II)	1.22	0.66 - 2.24	0.531	..	..	..
Treatment for inflammatory bowel disease†	..	..	..	..	..	..
Use of an antibiotic not directed at *C. difficile* infection during previous month						
Aminopenicillin	1.04	0.35 - 3.13	0.941	..	..	..
Aminopenicillin - β-lactamase inhibitor combination	1.17	0.60 - 2.28	0.643	..	..	..
Antipseudomonal penicillin - β-lactamase inhibitor combination	1.78	0.76 - 4.20	0.186	2.32*	0.79 - 6.82	0.125
Second-generation cephalosporin	0.62	0.26 - 1.43	0.261	..	..	..
Ceftazidime	2.25	1.17 - 4.29	0.015	2.48*	1.06 - 5.81	0.036
Any cephalosporin	1.11	0.63 - 1.94	0.721	..	..	..
Carbapenem	0.81	0.31 - 2.11	0.661	..	..	..
Aminoglycoside	1.60	0.59 - 4.28	0.354	..	..	..
Old quinolone	1.22	0.63 - 2.39	0.555	..	..	..
New quinolone	1.60	0.57 - 4.26	0.368	..	..	..
Any quinolone	1.35	0.73 - 2.47	0.335	..	..	..
Intravenous glycopeptide	1.73	0.71 - 4.20	0.228	..	..	..
Lincosamide	1.78	0.64 - 4.96	0.271	..	..	..
Macrolide	1.03	0.35 - 3.02	0.952	..	..	..
Co-trimoxazole	0.15	0.02 - 1.18	0.071	..	..	..
Episodes of *C. difficile* infection in 8 weeks before current episode	2.15	1.10 - 4.22	0.025	2.26*	1.03 - 4.96	0.041
***C. difficile* infection characteristics**						
Duration of diarrhoea >1 week	1.01	0.56 - 1.83	0.965	..	..	..
Diarrhoea mixed with blood	0.49	0.18 - 1.36	0.171	..	..	..
Fever (temperature >38.5°C)	1.17	0.71 - 2.06	0.572	..	..	..
Ileus	0.24	0.03 - 1.92	0.177	..	..	..
Leukocyte count ≥15 × 10^9/L	0.99	0.53 - 1.85	0.973	..	..	..
Serum creatinine rise >50%	0.90	0.30 - 2.69	0.850	..	..	..
Pseudomembranes‡	..	..	..	..	..	..
Ulceration	1.12	0.06 - 21.17	0.941	..	..	..
Colonic wall thickening	2.24	0.50 - 10.01	0.290	..	..	..
Pericolonic fat stranding	3.12	0.47 - 20.55	0.237	..	..	..
Bowel distension	0.60	0.16 - 2.24	0.445	..	..	..
Microbiological characteristics						
PCR-ribotype 027§	2.06	0.66 -6.43	0.211	..	..	..
PCR-ribotype 078§	1.62	0.67 -3.90	0.286	..	..	..
PCR-ribotype 014/020§	0.86	0.39 - 1.89	0.700	..	..	..
PCR-ibotype 015§	1.72	0.47 - 6.30	0.411	..	..	..
PCR-ibotype 018§	2.39	0.70 - 8.16	0.165	0.50¶	0.07 - 3.71	0.495
PCR-ibotype 023§	2.89	0.72 - 11.61	0.135	1.76¶	0.33 - 9.29	0.508
PCR-ibotype 056§	1.75	0.27 - 11.47	0.557	..	..	..
Presence of either or both binary toxin genes	1.63	0.89 - 2.97	0.113	..	..	..
Toxin A negative, toxin B positive strains *vs.* all other strains	0.69	0.14 - 3.46	0.654	..	..	..
Toxinotype III (including IIIb and IIIc) *vs.* all other toxinotypes	1.38	0.48 - 3.94	0.551	..	..	..

OR=odds ratio. APACHE II=acute physiology, age, chronic health evaluation version II. CHP=chronic health points. ..=data not available. *Adjusted for other: age ≥65 years, health-care association, pulmonary disease, chronic dialysis, previous use of antipseudomonal penicillin with β-lactamase inhibitor, previous use of ceftazidime, episodes of *C. difficile* infection 8 weeks before current episode, PCR-ribotype 018, PCR-ribotype 023, and presence of either or both binary toxin genes. †No recurrences in 19 patients with inflammatory bowel disease *versus* 83 recurrences in 419 patients without inflammatory bowel disease. ‡No recurrences in seven patients with pseudombranes *versus* two recurrences in 21 patients without pseudomembranes. §*Versus* all other ribotypes. ¶Adjusted for other variables: age ≥65 years, health-care association, pulmonary disease, chronic dialysis, previous use of antipseudomonal penicillin with β-lactamase inhibitor, previous use of ceftazidime and episodes of *C. difficile* infection 8 weeks before current episode.

Discussion

We have shown that the incidence of *C. difficile* infection and the distribution of causative PCR ribotypes differed greatly between hospitals in Europe; overall and attributable mortality were strikingly high. The strengths of this pan-European study are the large number of participating countries and hospitals, and a study design with a fixed 3-month follow-up. The high follow-up rate and the fact that patients with missing follow-up were younger, were more likely to be outpatients, and had less comorbidity than patients with follow-up, minimised the risk that cases of complicated infection were missed. If all patients with missing follow-up information had had an uncomplicated course, this factor would not have affected predictors for complicated infection.

This study has some limitations. First, selection of the hospitals in each country was left to the national coordinators, and the number of hospitals per country was small. Therefore, results derived from this sample of hospitals might not be representative of each country. Furthermore, some hospitals might have been selected because of outbreaks of *C. difficile* infection, thus introducing bias. Second, there might have been differences in physician awareness of infection between hospitals and countries. We note that the frequency of testing for infection varied up to 47 times between countries (as expressed by number of patients tested per 10,000 patient-days; table 1). Additionally, because there is no consensus on optimum testing for *C. difficile* infection, diagnostic (and culture) methods were not uniform. Third, detailed information for cases of infection was obtained only for the first ten patients enrolled in each hospital, which might have introduced bias if risk factors varied across hospitals. Furthermore, this low number might have led to under-representation of PCR ribotypes that caused outbreaks of infection in some hospitals.

Results from endoscopy or CT might be biased since these examinations tend to be triggered by a more severe course of disease. The proportion of patients with severe comorbidity might be overestimated because one of five items was sufficient to declare severe comorbidity, whereas if one item was scored missing, absence of severe comorbidity could not be declared.

Barbut and colleagues[21] reported a mean incidence of nosocomial *C. difficile* infection in 23 European hospitals of 2.45 per 10,000 patient-days (minimum to maximum range; 0.1-7.1), which is lower than the overall figure of 4.1 per 10,000 patient-days in our study. However, that study differed from ours in methodology. Reports from Denmark, Finland, Germany, Spain, and the UK[22–25] support the impression of an increase in incidence of *C. difficile* infection in Europe. PCR ribotypes identified by Barbut and colleagues[21] differed strikingly from those we identified. In their study, among isolates from 38 hospitals in 14 countries, PCR-ribotypes 001 and 014 were the most prevalent, followed by 027 and 020. Epidemic PCR-ribotype 027 was less prevalent in our study. By contrast, the prevalence of PCR-ribotypes 078 and 018 was increased. The high prevalence of PCR-ribotype 018 in our study is accounted for by its high prevalence in three Italian hospitals. Barbut and colleagues[21] reported that PCR-ribotype 078 was dominant only in Greece, whereas in our study it was the third most prevalent PCR ribotype. This increase of PCR ribotype-078 in Europe accords with findings for the Netherlands[26] and reports of PCR ribotype-078 in piglets with diarrhoea in the Netherlands and Spain.[27,28] Interestingly, human and animal isolates of PCR-ribotype 078 are genetically highly related, supporting the hypothesis that no interspecies barrier exists for *C. difficile* infection due to PCR-ribotype 078.[26] Research suggests that food products might play a part in interspecies transmission.[29,30] In one study, patients infected with PCR-ribotype 078 were younger than those infected with PCR-ribotype 027, but had a similar attributable mortality.[27] We could not show an association between PCR-ribotype 078 and complicated infection; however, patients with infection as a result of this ribotype (n=31) were more likely to have a rise in serum creatinine than were patients with other ribotypes (n=362, OR 3.20, 95% CI 1.08 - 9.49), and had a slightly higher mean leukocyte count.

Although we emphasise that *C. difficile* infection incidence rates of participating hospitals were not representative of national incidence rates, many hospitals with high rates of *C. difficile* infection were from countries in northern and central Europe. Most of these countries are thought to have low antibiotic consumption per head, even during the winter-respiratory-infection season.[31] Heightened awareness of *C. difficile* infection, as shown by the number of patients tested per 10,000 patient-days, might partly account for these differences in infection-incidence rates. Differences in the severity of illness of patients in hospital or those prescribed antibiotics might be other explanations. Patients admitted to high-incidence hospitals were more likely to have received aminopenicillins and first-generation and second-generation cephalosporins than were patients admitted to low-incidence hospitals.

Most risk factors for complicated or recurrent infection were consistent with those reported in previous studies. Old age,[32-34] previous hospital or nursing-home admission,[33] ileus,[33,34] and infection by PCR-ribotype 027[35] have been associated with complicated *C. difficile* infection. The use of certain antibiotics, especially fluoroquinolones, has been associated with infection by PCR-ribotype 027, and through this association with complicated or recurrent disease.[35,36] We did not find an association between the use of fluoroquinolones and complicated or recurrent disease, possibly because of the small number of infections resulting from PCR-ribotype 027 in our study. Alternatively, some confounding effects in earlier studies—notably data for antimicrobial prescribing in outbreak settings that might overestimate *C. difficile* infection risk associated with specific antibiotics—were not

as likely in our study. An association of PCR-ribotypes 018 and 056 with complicated infection has not been reported before. However, the number of complicated infections for which these associations were based was small. Old age[32,37] and a long cumulative duration of previous episodes of *C. difficile* infection[38] have been identified as predictors of recurrent infection. We could not confirm leucocytosis[33,34,37,39] as a strong predictor of complicated infection, possibly because we included leukocyte counts only from the week before the patients' inclusion, whereas in most studies the maximum leukocyte count during the course of the illness was examined. These findings underscore the importance of local surveillance to detect and control endemic and epidemic *C. difficile* infection.

Contributors

The study was designed by DWN, BHBB, MHW, and EJK, with support of DLM, on behalf of ECDC, and members of European Study group of *Clostridum difficile,* on behalf of European Society for Clinical Microbiology and Infectious Diseases. JSB and MR were responsible for PCR ribotyping and toxinotyping of strains, respectively. MPB did the study as principle coordinator, using support of DWN as principal investigator and EJK as microbiological coordinator. DLM helped in selecting national coordinators. BHBB and JTvD supervised clinical data collection and data analysis. MPB analysed the data and wrote the first draft of the article. All authors contributed substantially to the submitted version.

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Conflicts of interest

The authors declared no conflicts of interest.

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Chapter 5

Renal failure and leukocytosis are predictors of a complicated course of *Clostridium difficile* infection if measured on day of diagnosis

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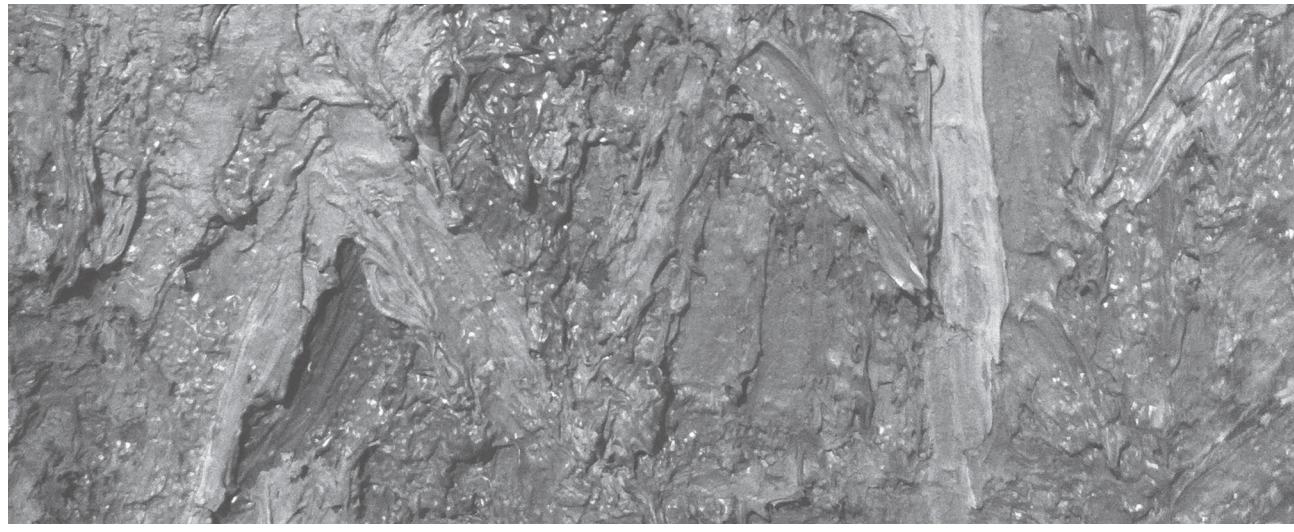

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Abstract

Nonsevere and severe *Clostridium difficile* infection (CDI), which carries a higher risk than nonsevere CDI for treatment failure and recurrence, are difficult to distinguish at the time of diagnosis. To investigate the prognostic value of 3 markers of severe CDI suggested by recent guidelines (fever, leukocytosis, and renal failure), we used the database of a randomized controlled trial, which contained information for 1105 patients with CDI. Leukocytosis (risk ratio [RR], 2.29; 95% confidence interval [CI], 1.63–3.21) and renal failure (RR, 2.52; 95% CI, 1.82–3.50) were associated with treatment failure. Fever, although associated with treatment failure (RR, 2.45; 95% CI, 1.07–5.61), was rare. Renal failure was the only significant predictor of recurrence (RR, 1.45; 95% CI, 1.05–2.02). Different timing of measurements of leukocyte count and serum creatinine around the CDI diagnosis led to a different severity classification in many cases. In conclusion, both leukocytosis and renal failure are useful predictors, although timing of measurement is important.

Introduction

Clostridium difficile infection (CDI) has become an increasing problem in many hospitals in the Western world during the past decade. *C. difficile* causes diarrhea and colitis with a tendency to recur after initially successful antimicrobial therapy. Furthermore, gut inflammation may be so severe that antimicrobial therapy is not effective; in such cases, complications such as hypotension, perforation, and toxic megacolon may develop. Several risk factors for CDI have been identified, of which the use of antibiotics is the most important. Predicting which patients are at risk for developing complications or recurrences can guide the choice and duration of therapy. In 2009, a prediction rule for recurrences, incorporating age, comorbid conditions, and the necessity to continue inciting antibiotic therapy, was published [1]. This rule was derived from and validated in 2 cohorts of 44 and 64 patients, respectively. The relatively small sample sizes challenge the credibility of this rule. Several risk factors for complications of CDI and prediction rules based upon these factors have been described, but unfortunately, none of these prediction rules have been validated [2–6].

The choice of an appropriate endpoint for a prediction rule for complicated and/or recurrent CDI has been problematic. The clinical judgment of whether to attribute endpoints such as CDI-related mortality and intensive care unit admission may be highly subjective, especially in elderly patients who are often admitted with severe illness and usually have significant comorbid conditions. Endpoints concerning the resolution and recurrence of diarrhea need a precise definition of diarrhea and quantitative measurement of stool volume and frequency, which may be difficult to obtain. Furthermore, the parameters included in a prediction rule should be objective, routinely measured in clinical practice, and be available at the moment the rule is applied (ie, when CDI is diagnosed).

A recent guideline by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) recommends that age, peak leukocyte count, and peak serum creatinine level be taken into account as potential indicators of a complicated course of CDI when treatment is started [7]. The European Society for Clinical Microbiology and Infectious Diseases (ESCMID) has issued a guidance document for the treatment of CDI that also lists qualitative and quantitative symptoms, signs, laboratory parameters, and radiological findings that may reflect more severe disease with associated higher risk for complications and recurrences [8]. Three quantitative parameters for diagnosing severe colitis were included: body temperature >38.5°C, leukocyte count >15 $\times$ 10^9/L, and serum creatinine level >50% above baseline; however, these cutoff values have not been confirmed prospectively.

In the present study, we sought to investigate the value of 3 quantitative severity criteria in predicting the failure of antimicrobial therapy and recurrence after initially successful treatment. Furthermore, we aimed to investigate whether leukocyte count and serum creatinine level fluctuate early in the course of a CDI episode and therefore whether the timing of their measurements can influence whether severity criteria are met. For our analyses, we used the database from 2 large randomized clinical trials that employed a strict objective definition of diarrhea and the database of a prospective single-center cohort study that recorded sequential leukocyte counts and serum creatinine levels around the date of CDI diagnosis.

Methods

Databases

The database from 2 randomized controlled phase 3 trials comparing vancomycin with fidaxomicin for the treatment of CDI was used to assess the predictive value of fever, leukocyte count, and serum creatinine level [9, 10]. Patients were recruited in the United States, Canada, and Europe (Study NCT00314951, April 2006–July 2008, United States, Canada; Study NCT00468728, April 2007–November 2009, United States, Belgium, Canada, France, Germany, Italy, Spain, Sweden, United Kingdom; www.clinicaltrials.gov). Patients with CDI, defined as diarrhea (>3 unformed bowel movements [UBMs] per day) with a positive stool toxin test result for *C. difficile*, were randomly assigned to receive vancomycin, 125 mg, 4 times daily or fidaxomicin, 200 mg, twice daily for 10 days. The number and times of UBMs were recorded during treatment and for 2 days after an end-of-therapy visit. For patients with rectal collection devices, volume was converted to number of UBMs by dividing the volume by 60 mL and rounding up to the nearest whole number. At the end-of-therapy visit, an investigator assessed the success of therapy. Clinical failure was defined as the persistence of diarrhea, need for additional therapy for CDI, or both, in the opinion of the investigator [10]. Recurrence of CDI (determined by use of the same criteria as for enrollment [ie, >3 UBMs per 24 hours and positive stool toxin test result]) was assessed during the 28 (±2) days of follow-up after completion of therapy. At enrollment, temperature, leukocyte count, and serum creatinine level were collected.

To assess whether the timing of laboratory measurements could influence their prognostic value, we used the database of a prospective cohort study performed at Leeds Teaching Hospital in 2007. In this database, 104 consecutive adult in-patients with CDI (unformed stool and positive *C. difficile* toxin test result) were included. On days −3 to +3 relative to day 0 (the day the diarrheal sample was collected), leukocyte count and serum creatinine level were recorded. A minimum of 2 leukocyte counts and creatinine levels on different days were required for patients to be included in the analyses.

In both analyses, we defined fever as core body temperature >38.5°C and leukocytosis as leukocyte count >15 × 10^9/L. Because the pre-CDI serum creatinine level was not known for each patient, we substituted the 50% creatinine level increase with a fixed value of the creatinine level >133 μmol/L (>1.5 mg/dL). This served as a proxy for renal failure.

Analyses

The intention-to-treat population that received at least 1 dose of study medication was used for the analysis. Distributions of the continuous variables of temperature, leukocyte count, and creatinine level were compared for patients with and without clinical treatment failure and recurrence. Non-normally distributed variables were compared with a Mann-Whitney *U* test. Proportions were compared with **β** test. Risk ratios (RRs) and 95% confidence intervals (CIs) were calculated for the associations of fever, leukocytosis, and renal failure with the outcome parameters. Kaplan-Meier survival curves were constructed to investigate the association of fever, leukocytosis, and renal failure with time to resolution of diarrhea (expressed in hours from the first dose of fidaxomicin or vancomycin). The log-rank test was used to test the difference between the survival curves. Cox regression was used to calculate hazard ratios (HRs) with 95% CIs. Receiver operating characteristic curves were constructed to assess the validity of the cutoff values used to define categorical variables. Variability of leukocyte counts and serum creatinine levels were compared within patients and expressed in absolute differences. All analyses were carried out in SPSS for Windows software, version 17.0 (SPSS Inc, Chicago, Illinois, USA).

Results

There were 1105 patients with CDI in the clinical trial database. Patients treated with vancomycin (566) or fidaxomicin (539) had similar median values for temperature, leukocyte count, and serum creatinine level and were evenly distributed across the groups based on dichotomized continuous variables (data not shown). Fever was rare; only 1.2% of patients (13 out of 1102) had a temperature >38.5°C. Median treatment duration was 11 days for the fidaxomicin and vancomycin treatment groups. Overall, 143 patients (13%) experienced clinical treatment failure at the end of treatment. Of the 962 patients who were cured after treatment, 194 patients (20%) experienced recurrence within the following 28 (±2) days.

Median leukocyte count and creatinine level were significantly higher in patients with clinical treatment failure; temperature distributions in patients with and without treatment failure were almost identical. In addition, dichotomous categories of fever, leukocytosis, and renal failure all showed significant correlation with treatment failure

(**Table 1**). Median creatinine level was significantly higher in patients with recurrence, and this parameter was the only significant predictor of recurrence (**Table 2**). Different cut-off values for the continuous variables of temperature, leukocyte count, and creatinine level, assessed by receiver operating characteristics, did not lead to higher relative risks and therefore better performance in the prediction of clinical treatment failure or recurrent CDI.

The probability of resolution of diarrhea within 10 days of treatment was slightly lower in patients with renal failure compared with patients without renal failure (HR, 0.83; 95% CI, .68–1.02; **Figure 1**). Neither fever (HR, 1.08; 95% CI, 0.61–1.91) nor leukocytosis (HR, 1.02; 95% CI, 0.84–1.24) was associated with a lower probability of resolution of diarrhea. Although creatinine level distributions were similar between patients treated with fidaxomicin and vancomycin, we repeated the analysis of renal failure as a predictor of resolution of diarrhea stratified according to treatment group and found similar results (vancomycin: HR, 0.80 [95% CI, 0.61–1.05]; fidaxomicin: HR, 0.88 [95% CI, 0.66–1.19]). Because recurrences occurred less often in patients treated with fidaxomicin, the CI is widest in that group.

Table 1 Determinants of Clinical Treatment Failure

Continuous variables				
Variable	Outcome	Median	IQR	*P*[a]
Temperature (°C)	Failure	36.8	36.4–37.2	.180
	Cure	36.7	36.4–37.1	
Leukocyte count ($\times 10^9$/L)	Failure	10.5	6.8–17.4	.002
	Cure	8.9	6.5–12.1	
Creatinine (μmol/L)	Failure	80	62–150	.005
	Cure	71	62–97	

Categorical variables					
Variable	Category		Failure (n/N)	RR[b]	95% CI
Fever	Temperature	>38.5°C	4/13	2.45	1.07–5.61
		≤ 38.5°C	137/1089		
Leukocytosis	Leukocytes	>15 $\times 10^9$/L	38/153	2.29	1.63–3.21
		≤ 15 $\times 10^9$/L	90/829		
Renal failure	Creatinine	≥ 133 μmol/L	41/160	2.52	1.82–3.50
		<133 μmol/L	91/896		

CI, confidence interval; IQR, interquartile range; RR, risk ratio.
[a]*P* value for the comparison between patients with clinical treatment failure with those with clinical cure.
[b]RR for the association of the variable with failure.

Table 2 Determinants of Recurrence

Continuous variables				
Variable	Outcome	Median	IQR	*P*[a]
Temperature (°C)	No recurrence	36.7	36.4–37.1	.827
	Recurrence	36.7	36.4–37.0	
Leukocyte count ($\times 10^9$/L)	No recurrence	8.8	6.5–12.1	.276
	Recurrence	9.1	6.6–12.8	
Creatinine (μmol/L)	No recurrence	71	62–97	.008
	Recurrence	80	62–115	

Categorical variables					
Variable	Category		Recurrence (n/N)	RR[b]	95% CI
Fever	Temperature	>38.5°C	1/9	0.55	0.09–3.51
		≤ 38.5°C	192/952		
Leukocytosis	Leukocytes	>15 $\times 10^9$/L	22/115	1.00	0.67–1.50
		≤ 15 $\times 10^9$/L	141/739		
Renal failure	Creatinine	≥ 133 μmol/L	32/119	1.45	1.05–2.02
		<133 μmol/L	149/805		

CI, confidence interval; IQR, interquartile range; RR, risk ratio.
[a]*P* value for the comparison between patients with recurrence with those without recurrence.
[b]RR for the association of the variable with recurrence.

Clinical treatment failure rates were similar in the fidaxomicin and vancomycin treatment groups regardless of clinical status using the 3 severity factors. Recurrence was significantly more frequent following vancomycin treatment compared with fidaxomicin. In patients without renal failure, 93 of 402 (23.1%) patients cured by vancomycin therapy had a recurrence, whereas only 56 of 403 (13.9%) experienced a recurrence after successful fidaxomicin treatment ($P < .001$). In patients with renal failure at baseline, fidaxomicin therapy was associated with a 60% reduction in frequency of recurrences (8/54 [14.8%]) relative to vancomycin (24/65 [36.9%]; $P = .007$). Likewise, in patients categorized as having leukocytosis or severe CDI, the incidence of recurrence was more than double for patients cured with vancomycin compared with those treated successfully with fidaxomicin ($P < .01$ for each comparison).

Because leukocytosis and renal failure at the time of diagnosis were shown to be the strongest predictors, we investigated the stability of these parameters during a 6-day interval around diagnosis. In the population from the database of Leeds Teaching

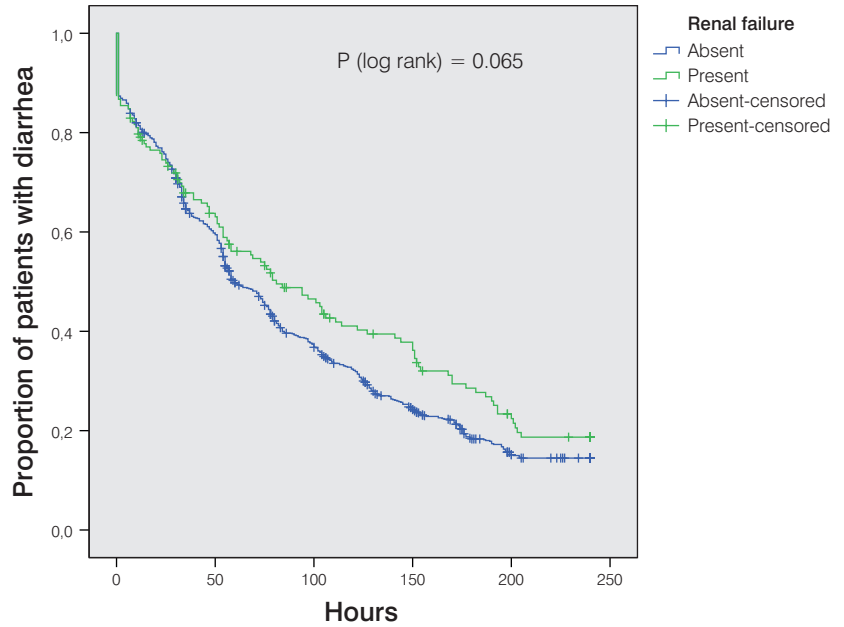

Figure 1 Kaplan-Meier analysis of time to resolution of diarrhea for patients with and without renal failure. The hazard ratio was 0.83 (95% confidence interval: 0.68–1.02).

Hospital, the highest mean leukocyte count was found on the day of CDI diagnosis (13.4×10^9/L). Within the interval from 3 days before to 3 days after the diagnosis of CDI, the mean difference between the highest and lowest leukocyte count values recorded was 6.4×10^9/L. Twenty of 86 (23.3%) patients had a minimum to maximum leukocyte count range $>10 \times 10^9$/L and 33 (38.4%) patients had a minimum to maximum leukocyte count range that included the cutoff of 15×10^9/L; therefore, a difference in timing of a single blood sample around diagnosis could have led to a different severity classification. Mean serum creatinine concentration was 147 μmol/L on the day of diagnosis. Mean minimum to maximum range in serum creatinine values was 38.7 μmol/L. Nineteen of 93 (20.4%) patients had a minimum to maximum creatinine range that included the cutoff of 133 μmol/L, which could have led to a different classification in the case of different timing.

Discussion

Leukocytosis and renal failure were significant predictors of failure of CDI treatment. Only renal failure showed a trend toward longer duration of diarrhea during treatment and was correlated significantly with recurrence after successful treatment. Both leukocyte count and serum creatinine level were highly variable around diagnosis. Fever was found to be too infrequent in our study to be a useful predictor, but its associated relative risk was significant.

In previous studies, leukocytosis and renal failure were also associated with complications and recurrence of CDI [3,11–13]. Therefore, both parameters could be suitable for evaluation in a prediction model. However, due to the variable nature of these values around the time of CDI diagnosis, a strict definition is needed before incorporating these parameters in a prediction rule. Early or late diagnosis could influence leukocyte count and serum creatinine level. Fever appeared not to be a useful predictor of failure of CDI treatment. This was also shown by a small study in 2007 [14].

Both fever and leukocytosis are thought to reflect more severe inflammation of the bowel wall. However, fever was too rare in our patient population to be of use as a predictor. Renal failure may reflect loss of effective circulating volume due to either dehydration because of diarrhea or shock in the context of a systemic inflammatory response. Unfortunately, the predictive value of these parameters may decrease because of underlying illnesses and comorbid conditions. Renal failure was present in 14% of clinical patients and was the only significant predictor of recurrence and the only parameter associated, albeit non-significantly, with a longer time to resolution of diarrhea. Thus, creatinine level may be good predictor, also because of its relatively greater stability around the time of CDI diagnosis in comparison to leukocytosis.

Strengths of this study are the large number of patients with CDI in the database with a well-described definition of diarrhea and a consistent measure of UBMs. Limitations include that other potential predictors of severe CDI, such as age, serum albumin level, or use of concomitant antibiotics, were not included in this analysis. Therefore, we were not able to develop a complete risk score. Another limitation is the absence of a baseline creatinine level for each patient, precluding us from distinguishing between chronic and acute renal failure.

The results of our study suggest that both leukocytosis and renal failure predict clinical treatment failure, whereas only renal failure is a predictor of recurrence after therapy. However, these predictors are highly dependent on the timing of their determination, hampering their use in clinical practice. We need better and more closely defined predictors to construct a reliable prediction score for complicated and recurrent CDI that is applicable in clinical practice.

Notes

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Potential conflicts of interest
S. L. G. is a part-time employee of Optimer Pharmaceuticals, receiving honoraria from and owning stock options in Cempra. M. M. is a consultant for Optimer Pharmaceuticals. D. N. G. holds patents licensed to ViroPharma for the treatment and prevention of CDI; is a consultant for ViroPharma, Optimer, Cubist, Merck, Pfizer, TheraDoc, Astellas, BioRelix, and Actelion; and holds research grants from GOJO, Merck, Optimer, Sanofi Pasteur, Eurofins Medinet, and ViroPharma. M. H. W. has received honoraria for consultancy work, financial support to attend meetings, and research funding from Astellas, Astra-Zeneca, Bayer, bioMerieux, Cerexa, Cubist, Nabriva, Novacta, Pfizer, Sanofi-Pasteur, Summit, The Medicines Company, and Viropharma. All other authors report no potential conflicts.

The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Chapter 6

Humoral immune response as predictor of recurrence in *Clostridium difficile* infection

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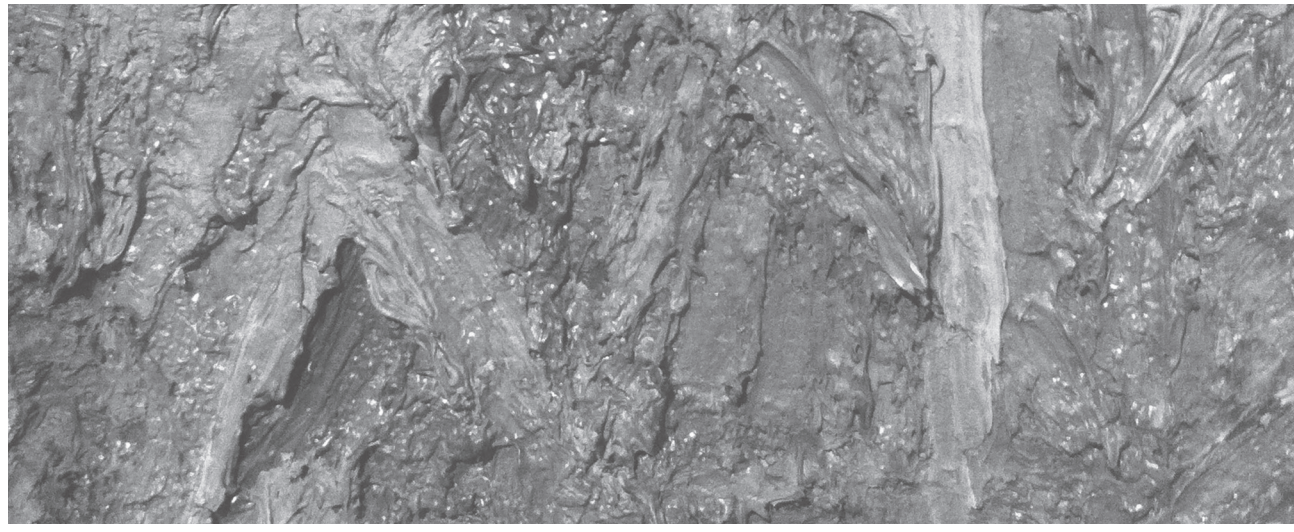

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Abstract

Low serum concentrations of antibodies directed against large clostridial toxins (TcdA and TcdB) have been associated with a higher risk of recurrence of *Clostridium difficile* infection (CDI) after successful antibiotic treatment. However, there are conflicting reports. Herein, we compared serum levels of antibodies of patients with a single episode of CDI with those of patients who subsequently suffered a recurrence. We used a serum bank from patients who received an experimental whey protein product following successful antibiotic treatment for CDI. We determined levels of IgA and IgG directed against TcdA, TcdB and non-toxin cell surface antigens in serum collected directly and three weeks after completing a 10 days course of antibiotic treatment for CDI. We also developed an objective flow cytometry-based assay to determine the proportion of cells exhibiting cytopathic effect after exposure to TcdB. Using this method, we measured sera's TcdB-neutralizing capacity. We compared the results for patients without a subsequent recurrence with those of patients who suffered a recurrence within 60 days after completing the antibiotic treatment. Advanced age, comorbidity other than immunocompromised state and low serum levels of anti-TcdA and TcdB antibodies were associated with recurrence, whereas serum levels of antibodies directed against cell surface antigens were not. Serum TcdB-neutralizing capacity, which correlated only weakly with serum IgG anti-TcdB, was not significantly associated with recurrence.

Key words: *Clostridium difficile*, toxins, antibodies, recurrence, IgA, IgG

Introduction

Clostridium difficile infection (CDI) is an important problem in healthcare facilities. Spores of this bacterium are ingested and bacteria may colonize the gut after germination. Colonization of the gut may lead to carriage or disease, which ranges from mild self-limiting diarrhea to fulminant life-threatening colitis. Mild disease may subside after withdrawal of antibiotics. Moderate and severe disease usually respond to oral metronidazole, glycopeptides, or fidaxomicin, but often recur. Factors associated with the outcomes of exposure to spores, colonization of the gut and disease include recent exposure to antibiotics, virulence of the *C. difficile* strain, advanced age, severe comorbidity, the use of proton pump inhibitors and the presence of antibodies directed against the large clostridial toxins TcdA and TcdB and/or other antigens [1].

However, the role of humoral immunity in CDI is unclear due to conflicting reports on the association between the humoral immune response and disease outcome [2-11]. Important issues include the type of antibodies (secretory IgA or IgG) involved, whether serum antibodies reflect mucosal immunity in the gut, whether TcdA or TcdB must be neutralized, which toxin epitope is the most important one, and whether antibodies directed against antigens other than toxin matter.

To investigate the possible relationship between the humoral immune response and the outcome of CDI, we compared serum levels of IgA and IgG directed against TcdA, TcdB and non-toxin cell surface antigens of patients with a single episode to those of patients with a subsequent CDI recurrence.

Methods

Serum samples

Serum samples were taken during a prospective cohort study [12] into the safety and preliminary efficacy of a whey protein concentrate made from milk of cows immunized against *C. difficile* to prevent recurrences after successful antibiotic treatment of CDI [13]. CDI was suspected clinically and confirmed by a positive fecal toxin assay and culture. Patient data registered on enrollment included age, sex, disease severity and chronic comorbidity according to the Acute Physiology, Age and Chronic Health Evaluation (APACHE) II prognostic system [14], previous episodes of CDI, and antibiotic treatment before starting the whey protein concentrate. After completion of 10 days of standard antibiotic therapy and reaching clinical remission of CDI, participating patients received this whey protein concentrate orally for two weeks with a follow-up period of 60 days. Outcome measures were CDI recurrences. Recurrence was declared if the patient reported looser stools according to a three-grade visual

scale, in comparison to the day before and an increase in stool frequency for two consecutive days, or a single day with an increase of ≥ 3 stools, or any day with passage of > 6 stools/day, and a positive *C. difficile* toxin stool test (Vidas, BioMérieux, Marcy l'Etoile, France) and culture. Among cultured strains, those strains belonging to PCR-ribotype 027 were identified as described earlier [15]. Serum samples were taken from these patients on the day they started on the whey protein concentrate (i.e., after 10 days of antibiotic therapy), and a second time 18 to 21 days later.

Enzyme-linked immunosorbent assay (ELISA) for the determination of serum anti-TcdA and anti-TcdB IgA and IgG

Wells of flat-bottom 96-well high-binding plates (Greiner Bio-One, Frickenhausen, Germany) were incubated with 100 μL per well of toxin in a 100 mM carbonate buffer (pH9.6) overnight at 4 °C. For the IgA antitoxin assays the wells were coated with 1.5 μg/mL of TdA [Mucovax, Leiden, Netherlands] or 1.0 μg/ml of TcdB [kindly provided by dr. H. Feng, Tufts University Cummings School of Veterinary Medicine, Grafton, MA] and for the IgG assays wells were coated with 1.0 μg/mL of TcdA or TcdB. The plates were then washed 3 times with phosphate-buffered saline (PBS; pH 7.4) as well as between every two incubation steps. Next, the plates were incubated with 200 μL of blocking buffer (2% wt/v gelatine in PBS containing 0.05% v/v Tween-20) for 1 hour at 37 °C. Thereafter, serial dilutions of serum samples diluted in 0.2% wt/v gelatine in PBS with 0.05% v/v Tween-20 were transferred to the plate (100 μL/well) and incubated for 90 minutes at 37 °C. All serum samples were tested in duplicate. The dilution buffer was used as a negative control. Subsequently, the plates were incubated with 100 μL of 4,000 x diluted polyclonal rabbit anti-human IgA antibodies conjugated with horseradish peroxidase (HRP) or 6,000 x diluted HRP-conjugated polyclonal rabbit anti-human IgG antibodies (DakoCytomation, Glostrup, Denmark) for 90 minutes at 37 °C. Lastly, the plates were incubated with 2.5 mg/mL of filtered 2,2'-azoni-bis(3-ethylbenzothiazoline-6-sulphonic acid) [ABTS] (Roche, Basel, Switzerland) in substrate buffer [47.2% of 100 mM citric acid and 52.8% of 100 mM Na_2HPO_4; pH 4.2] (100 μL/well) and 0.015% v/v H_2O_2 for 30 minutes at 37 °C. The absorbance was measured at 405 nm. For standardization, serum samples with high level of anti-TcdA immunoglobulins (IgA or IgG) were pooled, assigned an arbitrary value of 10,000 units (U) per mL, and used in all assays as standard.

ELISA for the determination of serum IgG directed against other antigens

The following non-toxin antigens were prepared from PCR ribotypes 001 and 027 as previously described: an EDTA extract representing the entire cell surface layer, a guanidine hydrochloride extracted S-layer proteins, an aqueous phenol extracted lipoteichoic acid (LTA) analogue (only from ribotype 001) [16] and whole flagella (mechanically sheared and purified on a cesium chloride gradient) as described by Hancock and Poxton [17]. ELISAs for IgG levels to these antigens were performed as described by Sanchez-Hurtado et al [16].

Flow cytometric assay to determine the capacity of serum to neutralize TcdB

We developed an objective method to determine the level of TcdB-neutralizing antibodies in sera. Briefly, 3T3 cells, a spontaneously immortalised cell line derived from embryonic mouse fibroblasts, were cultured in a 96-wells tissue culture plate until a semi-confluent monolayer was formed. Serial dilutions of TcdB in culture medium were preincubated 1:1 with fourfold diluted heat-inactivated patient serum for one hour at room temperature on a microplate shaker. Next, we incubated these mixtures of toxin and serum (100 μL per well) with the cells at 37 °C for one hour. Heat-inactivated pooled human serum (Lonza, Basel, Switzerland) was used as a standard. Toxin diluted in culture medium to a concentration of 50 ng/mL was used as a positive control and culture medium only as a negative control. After washings, the cells were cultured overnight and the degree of CPE was assessed microscopically. Next, we removed the cells from the wells by mechanically detaching them and then examined approximately 10,000 cells per sample on a FCSCalibur (Becton and Dickinson, La Jolla, CA). Results are expressed as the mean fluorescence intensity (MFI) in arbitrary units. The MFI of the cell population was used as a measure of the proportion of cells showing CPE. The MFI's for the positive control well and the negative control well were considered to characterize a cell population with maximum CPE and an unaffected cell population, respectively. For practical purposes, the mean of these MFI values was considered to represent 50% CPE. The MFI for the toxin dilution pre-incubated with patient serum that resulted in 50% CPE was divided by the value for the toxin dilution pre-incubated with standard serum resulting in 50% CPE to yield a standardized measure of the toxin-neutralizing capacity (Table 1).

Statistical analysis

The distributions of continuous variables were compared with a Mann-Whitney *U* test and proportions with the β test. The strength of the relationship between two continuous variables was estimated by Kendall's tau-b. Continuous variables were dichotomized using the median for the entire population as a cut-off. For the association between dichotomized variables and recurrence, odds ratios (ORs) with 95% confidence intervals were calculated. To examine the presence of confounding, categorical variables with a strong and clear association (OR > 2 and $P < 0.2$) with recurrence were introduced into a multivariate logistic regression model. IBM SPSS Statistics 20.0 software was used for the calculations.

Table 1 Comparison of the flow cytometric and light microscopical assessment of the toxic effect of TcdB on 3T3 cells.

In short, semi-confluent monolayers of 3T3 cells were exposed for 1 hour at 37 °C to various amounts of TcdB that had been pre-incubated with eight-fold diluted patient serum for 1 hour at ambient temperature (B-E), or as positive control to 50 ng/ml TcdB (A) and as negative control to medium alone (F). After washing and subsequent culturing overnight at 37 °C, the cytopathic effect (CPE) of the toxin was estimated by visual inspection or, after mechanical detachment of the cells from the wells, quantitated by flow cytometry, ie the median fluorescence intensity (MFI) of the forward scatter. We assumed that the mean of the MFI values for the positive control and the negative control (in this example: 409 arbitrary units [AU]) represents a cell population showing 50% CPE. The serum-preincubated TcdB concentration that resulted in a cell population with 50% CPE (in this example extrapolated to a TcdB concentration of 4.4 ng/L) is divided by the corresponding, pooled reference serum preincubated , TcdB concentration to yield a standardized measure of the TcdB-neutralizing capacity.

Treatment	MFI	Light microscopy
TcdB 50 ng/mL	310 AU	All cells showing CPE
TcdB 8.3 ng/mL with patient's serum	332 AU	More cells showing CPE than unaffected cells
TcdB 5.6 ng/mL with patient's serum	366 AU	Cells showing CPE mixed with unaffected cells
TcdB 3.7 ng/mL with patient's serum	436 AU	Cells showing CPE mixed with unaffected cells
TcdB 2.5 ng/mL with patient's serum	481 AU	More unaffected cells than cells showing CPE
Medium alone	508 AU	No cells showing CPE

[b]RR for the association of the variable with recurrence.

Results

Of 120 CDI patients whose data were present in the database, 16 (13.3%) suffered a toxin-confirmed recurrence. Table 2 shows patient characteristics. Advanced age and severe comorbidity were predictors of recurrence. However, immunocompromised state was not. Treatment with vancomycin was non-significantly associated with subsequent recurrence, probably reflecting more severe CDI for which treatment with vancomycin was preferred over metronidazole.

Table 2 Characteristics of CDI patients after completion of 10 days of antibiotic treatment

	Recurrence		No recurrence		
	N	Result	N	Result	*P*
Epidemiological characteristics:					
Age (median and IQR)	15	77 (73 – 84)	99	69 (52 – 79)	0.057
Age > 73 years (%)	15	80	99	46	**0.013**
Male sex (%)	16	38	103	50	0.371
Liver cirrhosis with portal hypertension* (%)	16	0	100	10	0.186
Heart failure NYHA class IV (%)	16	31	99	18	0.225
Severe pulmonary disease* (%)	16	44	98	28	0.189
Receiving chronic dialysis (%)	16	13	100	6	0.341
Immunocompromised* (%)	16	25	98	34	0.492
Any of the above comorbidities (%)	16	81	98	56	0.057
Any of the above comorbidities without immunocompromised status (%)	16	75	98	43	**0.017**
Recurrent CDI episode vs. first episode (%)	16	44	102	40	0.788
Number of previous CDI episodes (median andIQR)	16	0 (0 – 1)	102	0 (0 – 1)	0.839
Recent episode treated with metronidazole (%)	16	31	102	49	0.185
Recent episode treated with vancomycin (%)	16	69	102	48	0.123
Clinical, hematological, biochemical and microbiological characteristics:					
Leukocyte count [10^9/L] (median and IQR)	15	7.6 (6.1 – 15.3)	72	9.0 (6.6 – 13.3)	0.536
Creatinine [μmol/L] (median and IQR)	16	83 (61 – 124)	101	82 (62 – 127)	0.949
Serum albumin [g/L] (median and IQR)	13	38 (27 – 39)	66	31 (23 – 37)	0.092
APACHE II score (median and IQR)	12	2 (0 – 4)	57	1 (0 – 3)	0.380
Episode of CDI caused by PCR ribotype 027 (%)	12	50	60	28	0.142

* According to the Chronic Health Points score of APACHE II

CDI = *Clostridium difficile* infection. N = number of patients for whom information was available. IQR = interquartile range. Results printed in boldface have reached statistical significance ($P < 0.05$).

Table 3 shows IgA and IgG anti-TcdA and anti-TcdB antibody levels and TcdB-neutralizing levels in sera from patients with and those without recurrence. Low serum levels of IgA anti-TcdA and IgG anti-TcdB three weeks after completing 10 days of antibiotic treatment for CDI were most clearly associated with recurrence, as well as a decrease in serum IgG anti-TcdA during this time period. Some of these associations reached statistical significance. Interestingly, serum levels of anti-toxin A/B antibodies decreased in many patients during the three weeks after antibiotic treatment.

Low serum TcdB-neutralizing capacity was not a predictor of recurrence, although none of the patients with a higher serum neutralizing capacity than the reference serum suffered a recurrence. There was no correlation between anti-TcdB IgA level in serum and its capacity to neutralize TcdB (day 0: $r = 0.155$, $P = 0.185$; day 18: $r = 0.185$, $P = 0.129$) and only a weak correlation between anti-TcdB IgG level in serum and its capacity to neutralize TcdB (day 0: $r = 0.253$, $P = 0.036$; day 18: $r = 0.309$, $P = 0.011$).

Serum levels of IgG directed against any of the non-toxin cell-surface antigens after antibiotic treatment did not differ between patients with subsequent recurrence and those without subsequent recurrence (data not shown).

Antibody levels and neutralizing capacity were dichotomized using the median of the entire population of patients as a cut-off. Odds ratios for the association with recurrence were calculated (Table 4). To investigate possible confounding, the odds ratios for these dichotomized antibody levels were corrected for age over 73 and the presence of severe comorbidity. Serum levels of IgA anti-TcdB directly after antibiotic treatment and IgG directed against both toxins three weeks later and a decrease in serum IgG anti-TcdA were associated most strongly with recurrence.

Table 3 Antibodies against *C. difficile* antigens in serum of CDI patients with and those without recurrence

	Recurrence			No recurrence			
	N	Median	IQR	N	Median	IQR	*P*
Day 0:							
Serum IgA anti-TcdA [arbitrary units]	14	327	128 – 576	74	477	197 – 1027	0.133
Serum IgG anti-TcdA [arbitrary units]	14	580	162 – 1286	75	350	166 – 1430	0.696
Serum IgA anti-TcdB [arbitrary units]	15	986	659 – 1155	67	1603	665 – 2894	0.067
Serum IgG anti-TcdB [arbitrary units]	15	810	390 – 1423	65	974	537 – 3904	0.392
Serum TcdB-neutralizing capacity [proportion of that of reference pooled serum]	7	0.42	0.30 – 0.85	33	0.45	0.40 – 1.15	0.485
Day 18 – 21:							
Serum IgA anti-TcdA [arbitrary units]	14	183	0 – 389	76	423	198 –1255	**0.009**
Serum IgG anti-TcdA [arbitrary units]	14	237	0 – 815	75	486	186 – 1660	0.121
Serum IgA anti-TcdB [arbitrary units]	11	835	387 – 1001	68	1280	561 – 2551	0.133
Serum IgG anti-TcdB [arbitrary units]	11	446	224 – 892	68	954	527 – 2932	**0.030**
Serum TcdB-neutralizing capacity [proportion of that of reference pooled serum]	4	0.46	0.37 – 0.61	33	0.60	0.40 – 1.10	0.354
Increase between day 0 and day 18 – 21:							
Serum IgA anti-TcdA [arbitrary units]	14	-73	-372 – 2	74	-28	-297 – 66	0.620
Serum IgG anti-TcdA [arbitrary units]	14	-175	-795 – -20	74	0	-92 – 263	**0.007**
Serum IgA anti-TcdB [arbitrary units]	11	-36	-711 – 263	44	-451	-1485 – 83	0.323
Serum IgG anti-TcdB [arbitrary units]	11	-568	-2022 – 99	43	-358	-1007 – 263	0.499

CDI = *Clostridium difficile* infection. N = number of patients for whom information was available. IQR = interquartile range. Day 0 = the day after completing 10 days of antibiotic treatment. Results printed in boldface have reached statistical significance ($P < 0.05$).

Table 4 Dichotomized variables as predictors of CDI recurrence

	Recurrence		No recurrence					
	N	%	N	%	OR	95% CI	aOR	95% CI
Epidemiological characteristics:								
Age > 73 years	15	80	99	46	**4.80**	**1.28 – 18**		
Comorbidity other than immunocompromised status	16	75	98	43	**4.00**	**1.21 – 13**		
Antibody levels:								
Day 0:								
Serum IgA anti-TcdA ≤ 435 units	14	64	74	47	2.01	0.61 – 6.56	3.04	0.78 – 12
Serum IgG anti-TcdA ≤ 408 units	14	36	75	53	0.49	0.15 – 1.59	0.49	0.13 – 1.76
Serum IgA anti-TcdB ≤ 1271 units	15	80	67	43	**5.24**	**1.35 – 20**	**4.57**	**1.06 – 20**
Serum IgG anti-TcdB ≤ 949 units	15	53	65	49	1.18	0.38 – 3.63	1.39	0.39 – 4.98
Serum TcdB-neutralizing capacity ≤ 0.44	7	57	33	49	1.42	0.27 – 7.34	1.03	0.17 – 6.16
Day 18 – 21:								
Serum IgA anti-TcdA ≤ 366 units	14	71	76	46	2.93	0.84 – 10	**6.06**	**1.40 – 26**
Serum IgG anti-TcdA ≤ 460 units	14	64	75	48	1.95	0.60 – 6.37	3.31	0.85 – 13
Serum IgA anti-TcdB ≤ 1212 units	11	82	68	46	**5.37**	**1.08 – 26**	**6.53**	**1.05 – 40**
Serum IgG anti-TcdB < 854 units	11	73	68	47	3.00	0.73 – 12	3.25	0.65 – 16
Serum TcdB-neutralizing capacity < 0.60	4	75	33	52	2.82	0.27 – 30	4.50	0.25 – 81
Increase between day 0 and day 18 – 21:								
Serum IgA anti-TcdA ≤ -42 units	14	57	74	49	1.41	0.45 – 4.64	2.08	0.57 – 7.62
Serum IgG anti-TcdA ≤ -22 units	14	79	74	45	**4.56**	**1.17 – 18**	**5.35**	**1.22 – 23**
Serum IgA anti-TcdB ≤ -419 units	11	36	44	55	0.48	0.12 – 1.87	0.50	0.10 – 2.49
Serum IgG anti-TcdB ≤ -362 units	11	55	43	49	1.26	0.33 – 4.75	0.93	0.18 – 4.77

CDI = *Clostridium difficile* infection. N = number of patients for whom information was available. Day 0 = the day after completing 10 days of antibiotic treatment. OR = odds ratio. 95% CI = 95% confidence interval. aOR = odds ratio adjusted for age > 73 years and comorbidity other than immunocompromised status. Results printed in boldface have reached statistical significance (P < 0.05).

Discussion

The main findings of this study in patients with CDI are that advanced age, comorbidity and low serum levels of antibodies directed against TcdA and TcdB were associated with recurrence, whereas serum levels of antibodies directed against cell surface antigens were not.

The strong points of this study pertain to the study protocol and analytic methods. First, the data derive from a prospective study in which patient characteristics and sera were collected according to a standardized protocol. Second, blood was collected at two time points, which allowed for the analysis of the dynamics of antibody levels. Third, standardized ELISAs for antibodies against toxins A and B and an objective assay for toxin-neutralization capacity of sera were developed. Clostridial toxin neutralization assays are commonly based on microscopic assessment of cypathic effect (CPE) on cells. In these assays, a cell population showing 50% CPE is used to compare the rate of toxin neutralization. Because there are grades of CPE (e.g., loss of length and number of cell processes, gradual rounding of cells), the proportion of cells showing CPE may vary per visual field and certain unaffected cells that are not fully attached to the surface may appear to show CPE, we felt that assessing 50% CPE microscopically is subjective. Unfortunately, the relatively small number of patients with recurrence and the fact that all patients were treated with a whey protein concentrate containing antibodies against *C. difficile* and its toxins may have compromised statistical power to detect differences between CDI patients with recurrence and those without recurrence. Another limitation of the present study pertains to quantitation of the anti-toxin antibody levels in sera instead of fecal samples. Currently, it is not known to what extent serum antibody levels reflect mucosal immunity, which is probably the relevant part of the immune system given that CDI is not an invasive infection. Finally, the analysis of toxin-neutralizing capacity of the sera was limited to TcdB, as this toxin was considered the most important one in the pathogenesis of CDI according to scientific consensus at the time of experimentation.

Several studies have found associations between humoral immunity and the clinical course of CDI. For instance, it has been reported that serum IgG directed against TcdA, and less convincingly, IgG directed against TcdB and other antigens [5] as well as all classes of antibodies directed against whole cell *C. difficile* [8] are associated with disease instead of carriage. Warny [11] found low serum IgG and fecal sIgA directed against TcdA to be associated with a longer duration of illness and a higher risk of recurrence. Others reported that low levels of IgM directed against various antigens and low levels of IgG directed against TcdA, but not TcdB or other antigens predict recurrence [6]. Aronsson [2] identified low serum IgG directed against TcdB to be a better predictor of recurrence than low serum IgG directed

against TcdA. Leav [7] found IgG directed against the receptor-binding domain of TcdB and to a lesser extent that of TcdA, but not against whole toxins, and Drudy [3] reported IgM directed against surface-layer proteins to predict recurrence. By contrast, Johnson [4] and Sánchez-Hurtado [9] found that humoral immune responses did not influence the clinical course of CDI. Recently, Solomon [10] reported no correlation between serum IgG anti-TcdA and IgG anti-TcdB and recurrence, but did find a relationship between low levels of these antibodies and 30-day all-cause mortality, most of which was at least partly attributed to CDI.

Several findings raise the possibility that serum anti-toxin antibody levels are not causally related with recurrences. In this study, for example, the serum antitoxin antibody levels in most of the patients with and without recurrence decreased during the first three weeks after completion of the antibiotic course. In addition, immuno-compromised state was not associated with recurrence. Furthermore, in contrast to serum antibodies directed against toxins, the TcdB-neutralizing capacity of sera did not predict recurrence. It could be that low serum antibody levels are caused by fecal protein loss from a severely inflamed colonic mucosa. The severity of colonic inflammation may itself be associated with the risk of recurrence. The fact that several studies have found hypo-albuminemia to predict recurrence [18] and our study found patients with subsequent recurrence to recover less from hypo-albuminemia than patients without recurrence (data not shown) support this hypothesis. On the other hand, there are arguments against this hypothesis. Low levels of antibody against non-toxin, cell-surface antigens were not associated with recurrence. Moreover, the administration of parenteral monoclonal antibodies against the C-terminus of TcdA and TcdB has been found to prevent recurrences [19].

In conclusion, low serum anti-TcdA and anti-TcdB antibodies are associated with a higher risk of recurrence. However, further studies into the humoral immune responses in CDI, simultaneously measuring serum and fecal antibody levels at several time points, as well as measuring fecal protein loss (e.g., by fecal alpha1-antitrypsin clearance), may help to distinguish between a causal relationship and confounding.

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Chapter 7

Recidieven van *Clostridium difficile*-geassocieerde diarree voorkómen door toediening van een weiconcentraat van specifiek geïmmuniseerde koeien; prospectief onderzoek

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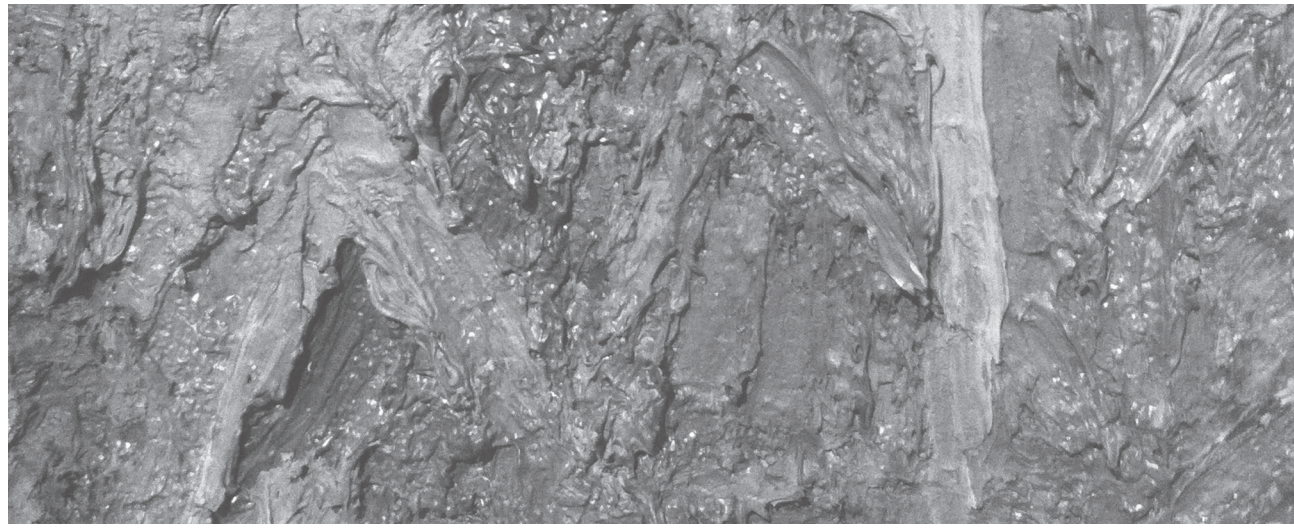

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Samenvatting

Doel. Wij probeerden recidiverende *Clostridium difficile*-geassocieerde diarree (CDAD) te voorkomen door behandeling met een 40%-wei-eiwitconcentraat van melk van koeien, geïmmuniseerd tegen *C. difficile* en zijn toxinen; dit weiconcentraat bevat specifiek neutraliserend secretoir IgA.

Opzet. Prospectief, niet-geblindeerd, klinisch cohortonderzoek.

Methode. In 2005-2006 kregen 100 patiënten met CDAD na een standaard antibiotische behandeling 2 weken weiconcentraat. Tot 60 dagen na de start van de toediening documenteerden wij aan de hand van zelfrapportage, bloedbepalingen en actieve surveillance eventuele bijwerkingen en het optreden van recidief-CDAD.

Resultaten. Het weiconcentraat werd goed verdragen en veiligheidsproblemen deden zich niet voor. In 11 van in totaal 109 ziekte-episoden (10%) trad een recidief-CDAD op. Na de behandeling met weiconcentraat bleek een positieve fecestoxine-uitslag of feceskweek van *C. difficile* samen te gaan met een recidief-CDAD (relatief risico respectievelijk: 8,2 (95%-BI: 1,04-64) en 4,7 (95%-BI: 0,5-47). Een positieve fecestoxine-uitslag tijdens de toediening van weiconcentraat ging samen met een vroeg CDAD-recidief.

Conclusie. In vergelijking met historische en contemporaine bevindingen bij controlegroepen leek weiconcentraat het optreden van een recidief-CDAD met circa 50% te verminderen. Waarschijnlijk schoot de standaarddosering weiconcentraat tekort om in alle episoden de *C. difficile*-toxinen in feces te neutraliseren.

Clostridium difficile-geassocieerde diarree (CDAD) ontstaat als bij een individu dat besmet is met sporen van de anaerobe grampositieve bacterie *C. difficile*, de sporen in de darm ontkiemen tot de vegetatieve vorm en toxinen gaan produceren. Het enterotoxine A en het cytotoxine B beschadigen darmslijmvlies en veroorzaken diarree. Daarbij kan het gaan om een gering ongemak, maar een pseudomembraneuze colitis of een toxisch megacolon kan ook voorkomen.[1]

De behandeling van patiënten met CDAD richt zich op het tenietdoen van de gevolgen van diarree, zoals dehydratie, en op eradicatie van de toxineproducerende bacteriën. Bij lichte vormen van CDAD kan het beëindigen van een uitlokkende antibiotische behandeling voldoende zijn om de diarree te stoppen.[2 3] Dit heeft als voordeel dat de residente flora van de darm niet verder verstoord wordt. Normaliter biedt darmflora bescherming tegen uitgroei van *C. difficile*, doordat de oppervlakte van de darm wordt afgeschermd en door de competitie om voedingsstoffen.

Als de patiënt een zieke indruk maakt of koorts en frequente of bloederige diarree heeft, zijn antibiotica geïndiceerd.[1 2] Het doel daarvan is de diarree in een kort tijdsbestek onder controle te brengen, dat wil zeggen voor klinische genezing te zorgen én de kans op het terugkeren van de diarree zo klein mogelijk te maken. Bij voorkeur dient een behandeling *C. difficile* uit de darm te elimineren; zo kan microbiologische genezing worden bereikt.

Frequente recidivering van CDAD. In een recente *Cochrane*-review is vastgesteld dat standaardbehandeling met vancomycine of metronidazol bij de meeste patiënten (> 90%) tot een verbetering van het symptomenbeeld leidt.[4] Maar bij ruim 20% van de patiënten treedt een recidief van CDAD op, meestal binnen 1-2 weken na het staken van het antibioticagebruik.[1 4] De kans op een recidief-CDAD blijkt hoger na infectie met de nieuwe epidemische stam *C. difficile* ribotype 027,[5 6] die ook Nederland is vastgesteld.[7] Terugkerende CDAD vormt een belangrijk gezondheidsprobleem, voor de patiënt die door aanhoudende diarree uitgeput raakt en ook voor de arts, die tevergeefs zal zoeken naar evidence-based richtlijnen om terugkerende CDAD te voorkómen. Verder zijn de verpleegkosten hoog.[8 9]

Immunisatie van koeien met *C. difficile*, enterotoxine A en cytotoxine B leidt tot specifiek secretoir IgA in de melk. Een 40%-concentraat van wei-eiwit van de melk van geïmmuniseerde koeien bevat een hoge concentratie van specifiek secretoir IgA.[10]

Wij onderzochten de toepassing van dat weiconcentraat voor passieve immuuntherapie bij patiënten met CDAD en tevens voor de preventie van een terugkerende episode van diarree.

Methoden en patiënten

Weiconcentraat gericht tegen *C. difficile*. Het weiconcentraat (40%) met polyklonale, specifieke secretoire IgA-antistoffen tegen *C. difficile* wordt gemaakt van de melk van koeien die geïmmuniseerd zijn met door formaldehyde gedode *C. difficile* en met geïnactiveerde toxinen uit een kweekfiltraat van *C. difficile*. Door een combinatie van nasale immunisatie en lokale immunisatie in de supramammaire lymfklieren van koeien met een stimulans gebaseerd op de toxigene *C. difficile*-stam VPI 10463, verkrijgt men een hoge concentratie van specifiek secretoir IgA in melk.[10] Ondanks deze hoge concentratie verschilt de totale hoeveelheid immunoglobuline niet van die in melk van niet-geïmmuniseerde koeien. Immunisatie beïnvloedt dus niet de hoeveelheid immuunglobulinen in de melk, maar de specificiteit. Uit de melk wordt volgens standaardmethoden uit de zuivelindustrie wei-eiwit gezuiverd, dat voldoet aan de Nederlandse kwalificaties voor samenstelling, microbiologische veiligheid en houdbaarheid van wei-eiwitconcentraat en consumptiemelk.[11] Het vloeibare product, in dit artikel verder aangeduid met 'weiconcentraat', wordt uitgevuld in sachets met elk 5 g. Antistoffen in het weiconcentraat neutraliseren in vitro de cytotoxische werking van *C. difficile*-toxinen en bieden in een diermodel met hamsters bescherming tegen darmontsteking door *C. difficile*.[10]

Patiënten en cohortonderzoek. In een prospectief klinisch open-labelcohort-onderzoek (Leids Universitair Medisch Centrum; protocolnummer: 2002.222) onderzochten wij het weiconcentraat. Wij includeerden patiënten met diarree en een positieve uitslag op de fecestoxinetest en een positieve feceskweek op *C. difficile*. Als exclusiecriterium gold een pre-existente darmaandoening, waardoor de interpretatie van de consistentie en de frequentie van de ontlasting problematisch zou zijn. Bij een specialistische diagnose "melkallergie" of "melkintolerantie" kon een patiënt evenmin deelnemen. Tenslotte moest de patiënt het product oraal of tenminste per maagsonde kunnen innemen.

Alle deelnemers ontvingen tenminste 10 dagen standaard antibiotische therapie voordat gestart werd met de toediening van weiconcentraat. De keuze van de antibiotische behandeling (metronidazol of vancomycine) werd overgelaten aan de behandelend arts. Het weiconcentraatpreparaat werd opgelost in mineraalwater en gedurende 14 dagen in een dagelijkse dosering van 15 g, verdeeld over 3 giften, ingenomen, zo mogelijk 1 h vóór de maaltijd. De standaarddosering weiconcentraat werd gekozen naar rato van de effectieve dosis in het gevalideerde proefdiermodel, op basis van literatuurgegevens en op basis van het eerste pilotonderzoek bij patiënten.[10]

Enkele dagen nadat de inname was beëindigd werd de patiënt bezocht, waarbij de therapietrouw werd beoordeeld door eventueel overgebleven sachets te tellen. Patiënten hielden tot 60 dagen nadat zij begonnen waren met de inname een dagboek bij, waarin zij behalve eventuele bijwerkingen ook de consistentie van de feces noteerden (aangeduid als "normaal", "semi-vast" of "waterig") en de frequentie van de ontlasting; deze gegevens waren bij de laatste 80 patiënten gedocumenteerd. Ook werden patiënten tussendoor bezocht en aan het einde van het onderzoek telefonisch geïnterviewd.

Onderzoeksuitkomsten. Als primaire onderzoeksuitkomst gold de veiligheid van het weiconcentraat. In dit verband werden bijwerkingen en complicaties gedocumenteerd; ook bepaalden wij een breed scala aan hematologische en biochemische bloedwaarden vóór en na inname van het weiconcentraat.

Een secundaire onderzoeksuitkomst was het optreden van een nieuwe episode van diarree (recidief) in de 60 dagen van de follow-up. Een klinisch recidief werd vastgesteld als de patiënt een verandering van de consistentie van de ontlasting rapporteerde (bijvoorbeeld van "normaal" naar "semi-vast") samen met een toename van de ontlastingsfrequentie over 2 opeenvolgende dagen of een toename van de ontlastingsfrequentie van 3 of meer keren op één dag; ook kon het gaan om elke dag dat de patiënt aangaf meer dan 6 keer ontlasting te hebben. Bij het vermoeden van een recidief bepaalden wij de toxinen in de feces en verrichtten wij de feceskweken op *C. difficile* (fecestoxinebepaling vond plaats met de "enzyme-linked fluorescent assay", VIDAS, BioMérieux, Marcy l'Etoile, Frankrijk).[12]

Onafhankelijk van de kwaliteit van de ontlasting verrichtten wij bij alle patiënten na het beëindigen van de behandeling met weiconcentraat een fecestoxinebepaling en feceskweek, namelijk op dag 14-18. Bij een groot deel van de patiënten (n = 78) deden wij dat ook bij het beëindigen van de follow-upperiode op dag 60.

Berekeningen. Vooraf werd geen formele poweranalyse verricht. De gegevens werden geanalyseerd met descriptieve statistische methoden; categorische variabelen werden vergeleken met de χ^2-toets.

Resultaten

Patiënten. In een periode van 2,5 jaar screenden wij 136 patiënten en namen wij er 101 op in het onderzoek (tabel 1). Aanvankelijk werden vooral patiënten uit de Leidse regio geïncludeerd, maar tijdens de nationale uitbraak van CDAD in 2005[8] ook uit andere plaatsen in het land, met name uit Amersfoort, Gouda en Den Haag. Veel patiënten waren bij de start van het onderzoek opgenomen in een zorginstelling; nacontroles konden bij de meesten door een researchverpleegkundige thuis plaatsvinden. Van de gescreende patiënten werden 35 niet in het onderzoek opgenomen, omdat er geen informed consent verkregen was (n = 30), omdat de patiënt overleed vóór de standaard antibiotische behandeling was afgesloten (n = 3), of op verzoek van de behandelend arts (n = 2).

Tabel 1 Kenmerken van 101 patiënten met *Clostridium difficile*-geassocieerde diarree (CDAD; 109 ziekte-episoden)*, die gedurende 2 weken behandeld werden met een weiconcentraat van immune koeien voor het voorkómen van een CDAD-recidief na standaard antibiotische therapie met vancomycine of metronidazol

kenmerk	
♂:♀	50:51
mediane leeftijd in jaren (interkwartielafstand)	74 (53-80)
mediane lengte in m (interkwartielafstand)	1,70 (1,66-1,79)
mediaan gewicht in kg (interkwartielafstand)	65 (58-80)
onderliggende aandoening; n†	
cardiovasculaire ziekte	21
longziekte	28
leverziekte	9
nierinsufficiëntie	6
immuungecompromitteerde status	31
“chronic health”-score; n†	101
score 0	44
score 2	8
score 5	49
episode van CDAD; n	109
eerste	65
een of meer recidieven	44
C. difficile; PCR-ribotype 027; n/n	24/106‡
behandeling van de laatste episode; n	109
vancomycine	58
metronidazol	48
combinatie van deze twee	3
laboratoriumwaarden bij aanvang van de behandeling met weiconcentraat; mediaan (interkwartielafstand)	
leukocytenaantal x 10^9/l	9,5 (7-14)
creatinine in μmol/l	85 (68-126)
albumine in g/l	30 (21-36)

* Van de patiënten overleed er 1 na inclusie, maar vóórdat het weiconcentraat ingenomen kon worden; van deze patiënt zijn de kenmerken hier wel opgenomen.
† Onderliggende aandoeningen en “chronic health”-score opgegeven volgens relevante onderdelen in het prognostisch “Acute physiology, age, chronic health evaluation”(APACHE)-scoringssysteem;[13] hoe hoger de score, hoe meer comorbiditeit.
‡ In 3 episoden was geen typering van de *C. difficile*-stam mogelijk.

De meeste geïncludeerde patiënten waren 65 jaar of ouder en hadden een duidelijke onderliggende medische aandoening; bijna de helft had een “chronic health”-score van 5 volgens het “Acute physiology, age, chronic health evaluation”-(APACHE)-scoringssysteem (zie tabel 1).[13]

Eén patiënt overleed na inclusie, maar vóór inname van het weiconcentraat. Zijn kenmerken staan wel in tabel 1, maar deze CDAD-episode werd bij de verdere berekeningen buiten beschouwing gelaten.

Bij de overgebleven 100 patiënten werd weiconcentraat toegepast tijdens in totaal 109 episoden van CDAD: 65 maal kreeg de patiënt het concentraat na een eerste episode en 44 maal na één of meer CDAD-recidieven; 1 patiënt nam 3 maal een kuur weiconcentraat en 7 patiënten deden dat 2 maal (zie tabel 1).

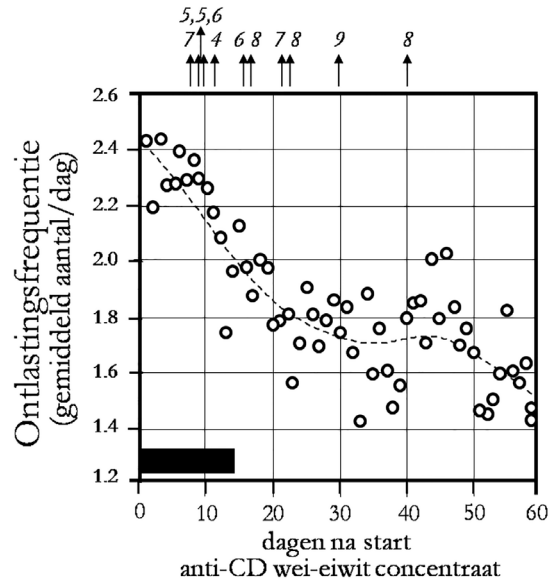
Veiligheid van het weiconcentraat en therapietrouw. 8 patiënten maakten de kuur van 14 dagen niet af: 4 vanwege de smaak van het concentraat, 1 vanwege een vroeg recidief, 1 patiënt omdat die kwam te overlijden aan een onderliggende medische conditie die niet aan de inname van het weiconcentraat was gerelateerd, en 2 patiënten omdat de arts alle medicamenteuze behandelingen bij hen beëindigde op grond van progressie van een onderliggende aandoening. Tijdens de 60 dagen follow-up overleden 5 patiënten door progressie van een onderliggende aandoening. In geen van deze gevallen was er naar het oordeel van de behandelende artsen een relatie met het weiconcentraat. Afgezien van de 4 deelnemers die afzagen van verdere inname op grond van de smaak werd het weiconcentraat goed verdragen.

Een uitgebreide screening van bloedparameters vóór en na het gebruik van het weiconcentraat en een evaluatie van de complicaties toonden geen negatieve invloed van het weiconcentraat. Het functioneren van de patiënten, afgemeten aan de karnofsky-score, en de gastro-intestinale kwaliteit-van-levenscore volgens de European Organisation for Research and Treatment of Cancer (EORTC)[14] verbeterden significant tijdens de inname van het concentraat. Gedetailleerde gegevens over de veiligheid van het weiconcentraat en de gecontroleerde bloedparameters zijn elders gepubliceerd.[11]

Op de 4 patiënten na (< 5%) die de inname staakten wegens onvrede met de smaak van het product bleek de innametrouw, afgemeten aan het ontbreken van overgebleven sachets, goed te zijn.

Recidiverende CDAD na gebruik van het weiconcentraat. Na de 10 dagen standaard antibiotische therapie was de gemiddelde ontlastingsfrequentie ondanks een verbetering van de symptomen bij alle patiënten nog steeds verhoogd, tot gemiddeld 2,4 keer per dag. In de loop van 4 weken nam de ontlastingsfrequentie af tot circa 1,7 keer per dag, waarna deze rond deze waarde stabiliseerde (figuur). De ontwikkeling van de fecesconsistentie liep hierop vooruit; de meeste mensen noemden de feces na circa 2-3 weken “semi-vast” tot “vast”. Kortom, kort na het beëindigen van een 10-daagse antibiotische behandeling waren de ontlastings-frequentie en -consistentie van veel patiënten nog niet genormaliseerd, en gemiddeld duurde het circa 4 weken voordat weer van een normaal ontlastingspatroon gesproken kon worden.

Bij 10 van de 100 patiënten werd aan de criteria voor een recidief-CDAD voldaan; 1 patiënt kreeg 2 maal een recidief en gebruikte in totaal 3 maal een kuur wei-



Figuur Dagelijkse gemiddelde frequentie van ontlasting bij patiënten met *Clostridium difficile*-geassocieerde diarree (CDAD) die werden behandeld met een weiconcentraat van tegen *C. difficile* geïmmuniseerde koeien. De behandeling duurde van dag 1-14 en is aangegeven met een balkje onder de horizontale as. Onder de grafiek staat de dagelijkse ontlastingsfrequentie tijdens 11 episoden die voldeden aan de definitie van "recidief-CDAD"; de pijlen geven het moment van het recidief aan.

concentraat; 7 patiënten met een recidief gebruikten het weiconcentraat 2 maal, 2 anderen zagen af van een 2e kuur. Er was dus in 11 van de 109 episoden (10%) sprake van een CDAD-recidief.

In 5 episoden trad het recidief op tijdens inname van het weiconcentraat (zie de figuur), in 2 episoden binnen 3 dagen na het staken van het concentraatgebruik en in 4 tijdens de follow-up, na respectievelijk 21, 22, 30 en 40 dagen. In alle gevallen van recidief lag de ontlastingsfrequentie evident buiten het groepsgemiddelde (zie de figuur) en was zowel de uitslag van de fecestoxinebepaling als van de feceskweek op *C. difficile* positief. Dit gold ook voor patiënten die tijdens de inname van weiconcentraat een recidief kregen.

De keuze van de voorafgaande standaard antibiotische behandeling (vancomycine versus metronidazol; zie tabel 1) hing niet samen met het percentage recidieven

($p > 0,25$). In 4 van de 24 episoden van CDAD door PCR-ribotype 027 trad een recidief op (17%), vergeleken met 7 van de 82 (9%) episoden veroorzaakt door een ander ribotype. De gemiddelde tijdsduur tot het optreden van een recidief verschilde overigens niet tussen infecties veroorzaakt door PCR-ribotype 027 of door andere ribotypen.

Recidief van CDAD gerelateerd aan fecestoxinebepaling en feceskweek. In 7 episoden trad een recidief op tijdens of vlak na de inname van weiconcentraat (zie de figuur). In deze episoden was de uitslag van de fecestoxinebepaling positief. Mogelijk was de dosering wei-eiwit onvoldoende om de werking van de toxinen compleet te neutraliseren en waarschijnlijk speelde dit een rol bij het optreden van het recidief. Deze hypothese werd gesteund door de bevinding dat een positieve fecestoxine-uitslag kort na het staken van de behandeling met weiconcentraat het optreden van een latere recidief-CDAD voorspelde (tabel 2). Na het voltooien van de behandeling met weiconcentraat hadden 12 van 96 patiënten nog een positieve fecestoxine-uitslag; van deze 12 kregen 2 een recidief (17%) tegenover slechts 2 van 84 patiënten (2%; relatief risico (RR): 8,20; 95%-BI: 1,04-64) met een negatieve fecestoxine-uitslag.

Tabel 2 Risico op een recidief van *Clostridium difficile*-geassocieerde diarree (CDAD) bij patiënten die gedurende 2 weken behandeld werden met een weiconcentraat van immune koeien, in samenhang met de uitslagen van bepalingen in feces van toxinen van *C. difficile* en van bacteriekweek. Vóór de behandeling met weiconcentraat hadden de patiënten standaard antibiotische therapie met vancomycine of metronidazol gekregen*

fecestest	aantal op dag 18-21	recidieven van CDAD; n/n (%)†	RR (95%-BI) van een positieve testuitslag voor recidief-CDAD
toxine-uitslag (n = 96)			
positief	12‡	2/12 (17)	8,2 (1,04-64)
negatief	84	2/84 (2)‡	
kweekuitslag (n = 98)			
positief	39	3/39 (8)	4,7 (0,6-47)
negatief	59	1/59 (2)‡	

RR = relatief risico.

* Er voldeden 7 patiënten aan de definitie van "recidief-CDAD" vóór dag 18; bij allen waren de toxine-uitslagen en de feceskweken positief op respectievelijk dag 7, 8, 8, 8, 10, 16 en 17.

† Er hadden 4 patiënten een recidief-CDAD op respectievelijk dag 21, 22, 30 en 40.

‡ Eén patiënt met een negatieve toxine- en kweekuitslag op dag 18-21, maar met toch een recidief-CDAD op dag 40, was opgenomen in een ziekenhuis waarin zich een epidemische verheffing van CDAD door ribotype 027 voordeed.

Eenzelfde verband gold voor de aanwezigheid van *C. difficile* in de feceskweek kort na het staken van de behandeling met weiconcentraat: in 3 van de 39 episoden (8%) met een positief kweekresultaat deed zich een recidief-CDAD voor, tegen 1 op 59 gevallen (< 2%) met een negatieve feceskweek (RR: 4,7; 95%-BI: 0,5-47). De 12 fecestoxinepositieve patiënten hadden ook een positieve feceskweek. Overigens, bij 1 patiënt met een laat recidief (op dag 40) was na het staken van de behandeling met weiconcentraat de toxine-uitslag negatief en was *C. difficile* niet aantoonbaar in de feceskweek. Deze patiënt was steeds opgenomen geweest op een afdeling met een epidemische verheffing van CDAD door ribotype 027 en mogelijk werd dit recidief veroorzaakt door een opnieuw exogeen verkregen *C. difficile* ribotype 027.

Van 78 patiënten zonder recidief waren feces, verzameld op dag 60, beschikbaar voor onderzoek. Bij 2 van hen bleek de fecestoxine-uitslag nog positief (2,6%; bevestigd in cytotoxiciteitstest) en bij 12 (15%) werd *C. difficile* geïsoleerd uit de feceskweek. Bij geen van deze 78 patiënten trad in het jaar na het onderzoek een recidief-CDAD op, hetgeen bleek uit telefonisch verkregen informatie.

Tenslotte, om beter te begrijpen of het weiconcentraat in de standaarddosering van 15 g per dag inderdaad compleet de *C. difficile*-toxinen in de darm neutraliseerde, werd bij 18 patiënten enkele dagen na de start van de behandeling nagegaan of de fecestoxine-uitslag negatief geworden was. Bij 4 patiënten (22%) was die uitslag nog positief. Bij 1 van deze 4 patiënten trad kort daarna een recidief-CDAD op, tegen géén van de 14 patiënten met een negatieve fecestoxine-uitslag tijdens inname van het weiconcentraat ($p < 0,05$).

Beschouwing

In dit prospectieve onderzoek leek de inname van het immune weiconcentraat na standaard antibiotische behandeling het optreden van CDAD-recidieven met circa de helft te kunnen verminderen. Deze conclusie berust op een vergelijking van het percentage recidief-CDAD in deze verkennende studie (10%) met gerapporteerde recidiefpercentages in de medische literatuur van 20-45 en met de Nederlandse gegevens over de uitbraak van 2005 met 25% recidieven.[5 6 15] Hierbij merken wij op dat ons verkennende prospectieve onderzoek niet geblindeerd was en dat de bevindingen nu bevestigd moeten worden door gecontroleerd klinisch onderzoek.

Het weiconcentraat bevat hoge concentraties van specifiek secretoir IgA gericht tegen *C. difficile* en zijn toxinen, en de werkzaamheid zou berusten op passieve immuuntherapie. Het weiconcentraat werd goed verdragen en de innametrouw was goed, zeker gezien de oudere patiëntengroep en de vaak ernstige onderliggende medische aandoeningen.

Er is in de literatuur een aantal meldingen van oraal gebruik van wei of koeien-immunoglobulinen. Die werden ingezet bij de behandeling of preventie van maag- of darminfecties, zoals met *Helicobacter pylori*,en bij diarree door enterotoxigene *Escherichia coli*, *Rotavirus* en *Shigella flexneri*.[16-19] Net als in ons onderzoek werden geen klinisch relevante bijwerkingen van orale inname van deze concentraten gemeld.

Er bestaat onduidelijkheid over de aanpak van terugkerende CDAD.[1 3 8 9] Bij de behandeling is gunstige ervaring opgedaan met specifieke schema's van vancomycine, waarbij het middel na standaardbehandeling over een aantal weken afgebouwd ('taper'-schema) respectievelijk intermitterend gegeven wordt ('pulse'-schema).[20] Maar elke behandeling met antibiotica, vancomycine en metronidazol inbegrepen, hoe noodzakelijk ook, vergroot de kans op het terugkeren van CDAD, omdat antibiotica de residente darmflora negatief beïnvloeden en *Clostridium*-sporen resistent zijn tegen antibiotica. Komt een patiënt dan ook eenmaal in een neergaande spiraal van terugkerende CDAD, dan wordt de kans op een recidief steeds groter en deze bedraagt uiteindelijk wel 60-70%.[8 9] Dit is de reden dat gezocht wordt naar alternatieve behandelingen, in plaats van of naast het gebruik van antibiotica (tabel 3). Een uitvoerige bespreking van deze alternatieven vindt men elders.[3 8 9]

Geconcludeerd kan worden dat er nog onvoldoende bewijs bestaat voor de werkzaamheid van verschillende behandelingen, met gerapporteerde succes-percentages die uiteenlopen van 30-100. Daarbij kan men denken aan intraveneuze infusie van immuunglobuline, bacteriotherapie of fecale transplantatie, toxineabsor-berende harsen of probiotica, maar ook aan de taper- en pulsebehandeling met vancomycine. De hypothese achter passieve immuuntherapie van CDAD met weiconcentraat is dat specifieke antilichamen de toxinen van *C. difficile* in de darm kunnen neutraliseren, en de vegetatieve vorm van de bacterie kunnen beletten zich aan de darmoppervlakte te hechten door afscherming van adhesiefactoren. Verdere beschadiging van darmslijmvlies wordt daarmee voorkomen zonder de residente darmflora negatief te beïnvloeden. De darm zal de sporen van *C. difficile* uiteindelijk zelf klaren. Voor deze hypothese bestaat dierexperimentele onderbouwing.[10] Daarnaast onderscheidt het gebruik van weiconcentraat zich van de meeste andere alternatieve behandelingen, doordat het pathogeenspecifiek is. De secretoir-IgA-antistoffen in het weiconcentraat zijn polyklonaal, zodat op meerdere plaatsen op de bacteriën en toxinen van *C. difficile* aangegrepen kan worden.[11] In principe zou het weiconcentraat ook toegepast kunnen worden in combinatie met antimicrobiële therapie, of preventief bij contacten tijdens een epidemische verheffing van CDAD.

Het dagelijks bijhouden van frequentie en consistentie van ontlasting laat zien hoe belangrijk het is een strikte definitie van "recidief-CDAD" te hanteren. Het is ons immers duidelijk geworden dat de meeste patiënten na het voltooien van de standaard antibiotische behandeling nog wekenlang een hogere ontlastingsfrequentie dan normaal en een afwijkende fecesconsistentie hadden. Bovendien was bij een hoog percentage (circa 10) van de patiënten aanvankelijk nog *C. difficile*-fecestoxine in de ontlasting aantoonbaar, zonder dat er sprake was van een recidief. Ofschoon een

Tabel 3 Niet-antibiotische behandeling van terugkerende *ClostridiumClostridium difficile*-geassocieerde diarree (CDAD): gerandomiseerde gecontroleerde klinische onderzoeken en observationele studies

1e auteur	behandeling	aantal patiënten	succes (in %)
gerandomiseerd, gecontroleerd klinisch onderzoek			
probiotica			
McFarland[21]	vancomycine of metronidazol + Saccharomyces boulardii 2 x 10^{10} KVE/d gedurende 4 wkn	26	65
	vancomycine of metronidazol + placebo	34	35
Surawicz[20]	vancomycine 500 mg 4 dd gevolgd door S. boulardii 2 x 10^{10} KVE/d gedurende 4 wkn	18	83
	vancomycine 500 mg 4 dd gevolgd door placebo gedurende 4 wkn	14	50
	vancomycine 125 mg 4 dd gevolgd door S. boulardii 2 x 10^{10} KVE/d gedurende 4 wkn	45	49
	vancomycine 125 mg 4 dd gevolgd door placebo gedurende 4 wkn	38	55
	metronidazol 1 g/d gevolgd door S. boulardii 2 x 10^{10} KVE/d, gedurende 4 wkn	27	52
	metronidazol 1 g/d gevolgd door placebo gedurende 4 wkn	26	50
Wullt[22]	metronidazol + Lactobacillus plantarum 299v 5 x 10^{10} KVE/ d gedurende 38 dgn	12	58
	metronidazol + placebo gedurende 38 dgn	9	33
Lawrence[23]	vancomycine of metronidazol gevolgd door Lactobacillus rhamnosus GG 6 x 10^{11} KVE/d gedurende 21 dgn	8	62
	vancomycine of metronidazol gevolgd door placebo gedurende 21 dgn	7	86
observationeel onderzoek			
Probiotica			
Gorbach[24]	metronidazol of bacitracine, 10 dgn, gevolgd door Lactobacillus GG 10^{10} KVE/d, 7-10 dgn	5	80
Biller[25]	L. rhamnosus GG 6 x 10^{9} KVE/d gedurende 14 dgn	4	100
bacteriotherapie en fecestransplantatie			
Bowden[26]	fecaal klysma	16	81
Tvede[27]	fecaal of bacterieel klysma	6	100
Lund-Tønnesen[28]	fecale instillatie door middel van coloscoop of via gastrostomie	18	83
Aas[29]	fecale instillatie door middel van maagsonde, circa 3 keer	16	94
Jorup[30]	fecaal klysma	5	100
Borody*	fecaal klysma	61	100
Lund*	fecale instillatie via jejunumsonde	20	83
Moore*	fecaal klysma	65	97
Aas*	fecale instillatie via maagsonde	9	100
passieve immuuntherapie met humane antistoffen			
Leung[31]	gammaglobuline 400 mg/kg i.v. elke 3 wkn gedurende 4-6 maanden	5	100
Beales[32]	gammaglobuline 400 mg/kg i.v. op d 1 en d 21	4	100
	gammaglobuline i.v., wisselend schema	5	60

KVE = kolonievormende eenheden; d = dag; dgn = dagen; wkn = weken.
**Onderzoek door J.Bakken gepresenteerd tijdens de bijeenkomst van de Infectious Disease Society of America in San Diego, 2007 (www.idsociety. org/content.aspx?id=7048#Oct_4).

dergelijke positieve uitslag een voorspellende waarde had voor het optreden van een recidief, herstelde de stoelgang bij de meeste patiënten uit deze groep spontaan. Bij enkele personen was de fecestoxine-uitslag zelfs bij het afsluiten van het onderzoek op dag 60 nog positief, zonder dat dit diarreeklachten gaf. Dit geeft aan dat toxinevormende *C. difficile* na een episode van CDAD nog lange tijd in de darm aanwezig kan zijn zonder klachten van een recidief te veroorzaken. Meer in het algemeen - en los van de huidige interventie - kan men stellen dat een positieve fecestoxine-uitslag na het beëindigen van antibiotische therapie voor CDAD dan ook geen aanleiding dient te zijn een herstellende patiënt direct opnieuw met antibiotica te gaan behandelen. Ook is het de vraag of een dergelijke bepaling routinematig, buiten onderzoeksverband om, uitgevoerd dient te worden.

De dosering van het weiconcentraat kwam op enigszins arbitraire gronden tot stand, en werd onder andere afgemeten aan de dosering gebruikt in een gevalideerd diermodel en aan eerste gegevens bij patiënten.[15 21-23 33] Dit onderzoek toont aan dat, als men ervan uitgaat dat het weiconcentraat altijd werkzaam is, bij een klein deel van de patiënten met CDAD een dagelijkse inname van 15 g van weiconcentraat te gering was om *C. difficile*-toxinen in de feces volledig te neutraliseren. De kans op het optreden van een recidief-CDAD was groter als de fecestoxinebepaling tijdens de inname van weiconcentraat nog een positieve uitslag had. Hetzelfde gold als kort na het staken van het weiconcentraatgebruik de uitslag weer positief werd. Het is daarom aannemelijk dat de effectiviteit van de aanpak vergroot wordt door in zulke gevallen de dosering weiconcentraat te verhogen. Dit is mogelijk door enkele dagen na de start van het gebruik van weiconcentraat of kort na het staken hiervan, een fecestoxinebepaling te verrichten. Als die positief uitvalt, kan de dosering van het weiconcentraat worden verhoogd of de inname worden hervat. Voor deze toepassing is nu een 80%-concentraat van het wei-eiwit ontwikkeld, dat bovendien lactosearm is. Inmiddels hebben wij bij enkele CDAD-patiënten ervaring opgedaan met zo'n op de persoon afgestemde aanpak. Hierbij bleek dat na verhoging van de dosering wei-eiwit de fecestoxine-uitslag inderdaad alsnog negatief wordt. Binnenkort zal een prospectief klinisch onderzoek starten om de effectiviteit van een geïndividualiseerde aanpak in een grotere patiëntengroep te bevestigen.

Conclusie

Onze eerste ervaringen met een weiconcentraat van koeien geïmmuniseerd tegen *C. difficile* en zijn toxinen tonen dat het preparaat veilig gebruikt kan worden bij patiënten met CDAD voor de preventie van terugkerende episoden van diarree. De dosering moet nader onderzocht worden. De gunstige bevindingen van ons verkennende, ongeblindeerde, prospectieve onderzoek dienen nu bevestigd te worden in gecontroleerde studies.

De toepassing van anti-CD-wei-eiwit-40%-concentraat en het klinisch onderzoek werden mogelijk gemaakt door niet-gerestringeerde financiële ondersteuning van MucoVax BV, Leiden.

Belangenconflict: geen gemeld. Financiële ondersteuning: geen gemeld.

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Abstract

Recurrence of *Clostridium difficile*-associated diarrhoea prevented by the administration of a whey concentrate from specifically immunised cows; prospective study

Objective. To try to prevent recurrences of *Clostridium difficile*-associated diarrhoea (CDAD) by treatment with a specific neutralising secretory IgA-enriched whey-protein concentrate (40%) made from the milk of cows immunised with *C. difficile* and its toxins.

Design. Prospective, non-blinded, clinical cohort study.

Method. In 2005-2006, 100 consecutive patients with CDAD received the whey concentrate for 2 weeks after completion of standard antibiotic therapy. For a period of 60 days after the start of the administration, the safety and preliminary efficacy of the whey concentrate were evaluated by means of a diary, blood determinations, active surveillance for adverse events, and the recurrence of CDAD.

Results. The whey concentrate was well tolerated and no safety issues were raised. Eleven out of 109 episodes (10%) were followed by a recurrence. After completion of the whey concentrate therapy, a positive test for faecal toxins or culture of *C. difficile* was predictive for the recurrence of CDAD (relative risk: 8.2 (95% CI: 1.04-64), and 4.7 (95% CI: 0.5-47), respectively). A positive faeces toxin during administration of the whey concentrate was also associated with an early recurrence of CDAD.

Conclusion. Compared to historical and contemporary findings in control groups, the whey concentrate appeared to reduce the recurrence of CDAD by about 50%. However, the standard dose of the whey concentrate was probably not sufficient to fully neutralise the *C. difficile* toxins in faeces in all episodes.

Chapter 8

European Society of Clinical Microbiology and Infectious Diseases (ESCMID): treatment guidance document for *Clostridium difficile* infection (CDI)

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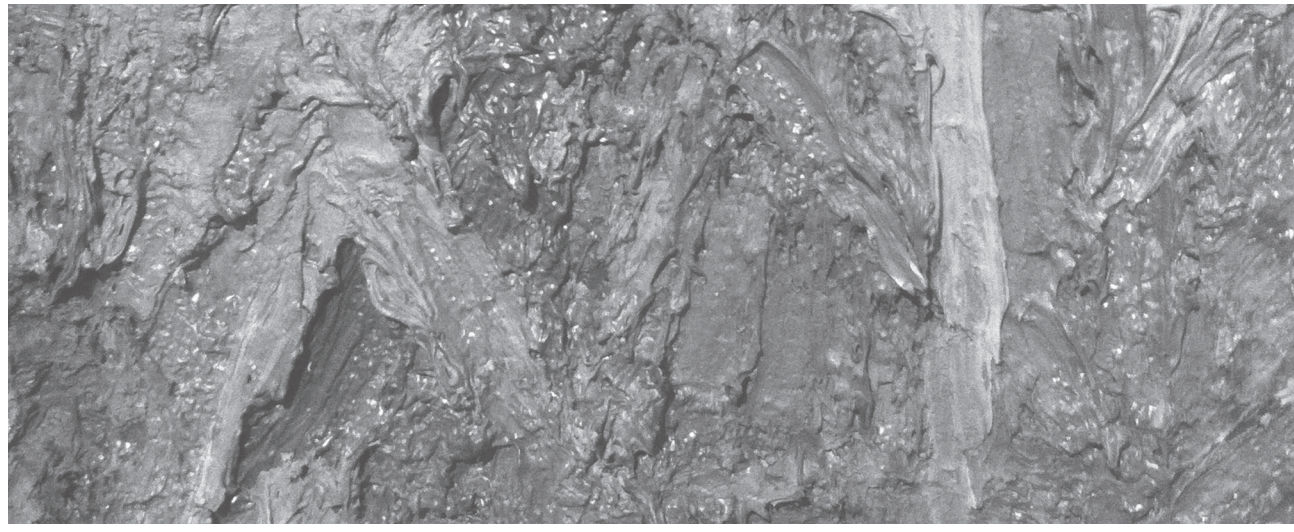

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Abstract

Clostridium difficile infection (CDI) is a potentially fatal illness with an increasing incidence worldwide. Despite extensive ongoing research into CDI treatment, management of CDI still poses important problems, such as a high propensity to relapse and refractoriness to treatment, especially when there is an ileus and oral drugs cannot be adminstered. This guideline evaluates the available literature, discusses criteria for disease severity and provides recommendations for CDI treatment, indicating level of evidence and strength of recommendation.

Keywords: *Clostridium difficile*, treatment, guideline

Summary of definitions and recommendations

Definitions

Episode of CDI =

1. a clinical picture compatible with CDI and microbiological evidence of toxin-producing *Clostridium difficile* in stool without evidence of another cause of diarrhoea or
2. pseudomembranous colitis (as diagnosed during endoscopy, after colectomy or on autopsy)

Clinical pictures compatible with CDI:

1. diarrhoea =
   a. loose stools, i.e. taking the shape of the receptacle or corresponding to Bristol stool chart types 5 to 7 and
   b. a stool frequency perceived as too high by the patient
2. ileus =
   a. signs of severely disturbed bowel passage such as vomiting and absence of stool and
   b. radiological signs of bowel distension
3. toxic megacolon =
   e. radiological signs of distension of the colon and
   f. signs of a severe systemic inflammatory response

Signs of severe colitis:

- fever (core body temperature $> 38.5\ ^{o}C$)
- rigors (uncontrollable shaking and a feeling of cold followed by a rise in body temperature)
- hemodynamic instability including signs of septic shock
- signs of peritonitis, including decreased bowel sounds, abdominal tenderness, rebound tenderness and guarding
- signs of ileus, including vomiting and absent passage of stool
- marked leukocytosis (leukocyte count $> 15 \cdot 10^{9}/l$)
- marked left shift (band neutrophils > 20% of leukocytes)
- rise in serum creatinine (>50% above the baseline)
- elevated serum lactate
- pseudomembranous colitis (endoscopy)
- distension of large intestine (imaging)
- colonic wall thickening including low-attenuation mural thickening (imaging)
- pericolonic fat stranding (imaging)
- ascites not explained by other causes (imaging)

Severe CDI =
an episode of CDI with one or more signs of severe colitis.
CDI without signs of severe colitis in patients with high age (≥ 65), serious comorbidity, ICU admission, or immunodeficiency may be regarded as severe.

CDI treatment response =
1. stool frequency as perceived by the patient decreases or stool consistency improves after three days and
2. no new signs of severe colitis develop

CDI treatment failure =
absence of CDI treatment response

CDI recurrence =
1. stool frequency as perceived by the patient increases for two consecutive days and stools become looser or new signs of severe colitis develop and
2. microbiological evidence of toxin-producing *C. difficile* in stool without evidence of another cause of diarrhoea after an initial CDI treatment response

Recommendations (implementation category between brackets)
1. Antiperistaltic agents and opiates should be avoided. (B-II)
2. In general, strive to use antibiotics covering a spectrum no broader than necessary and narrow the antibiotic spectrum of treatment after results of cultures and/or susceptibility tests become known. (B-III)
3. Mild CDI (stool frequency < 4 times daily; no signs of severe colitis), clearly induced by the use of antibiotics, may be treated by stopping the inducing antibiotic. Observe patients closely for any signs of clinical deterioration and place on therapy immediately if this occurs. (B-III)
4. Treatment for an initial episode and a first recurrence of CDI:
   If oral therapy is possible:
   - non-severe: metronidazole 500 mg tid orally for 10 days (A-I)
   - severe: vancomycin 125 mg qid orally for 10 days (A-I)

   If oral therapy is impossible:
   - non-severe: metronidazole 500 mg tid intravenously for 10 days (A-III)
   - severe: metronidazole 500 mg tid intravenously for 10 days (A-III) + intracolonic vancomycin 500 mg in 100 ml of normal saline every 4 – 12 h (C-III) and/or vancomycin 500 mg qid by nasogastric tube (C-III)
5. Colectomy should be performed to treat CDI in any of the following situations:
   - perforation of the colon
   - systemic inflammation and deteriorating clinical condition not responding to antibiotic therapy; this includes the clinical diagnoses of toxic megacolon and severe ileus. Colectomy should preferably be performed before colitis is very severe. Serum lactate may, inter alia, serve as a marker for severity (operate before lactate exceeds 5.0 mmol/l).
6. Treatment for a second recurrence of CDI and later recurrences:
   If oral therapy is possible:
   - vancomycin 125 mg qid orally for at least 10 days (B-II)
   - consider a taper (for example, decreasing daily dose with 125 mg every 3 days)/pulse (for example, a dose of 125 mg every 3 days for 3 weeks) strategy (B-II)

   If oral therapy is impossible:
   - metronidazole 500 mg tid intravenously for 10 – 14 days (A-III) + retention enema of vancomycin 500 mg in 100 ml of normal saline every 4 – 12 h (C-III) and/or vancomycin 500 mg qid by nasogastric tube (C-III)
7. In all the above-mentioned cases, oral vancomycin may be replaced by teicoplanin 100 mg bid, if available.

Introduction

Clostridium difficile infection (CDI) may arise when a patient's bowel is colonized by *C. difficile* after ingestion of spores, the spores subsequently germinate and the vegetative bacteria start producing toxins. Colonization is inhibited by the normal intestinal flora, which is hypothesized to compete with *C. difficile* for nutrients and space on the mucosal surface. Therefore, the use of antibiotics is the most important risk factor for CDI. The vegetative state of the bacterium is resistant to a varying but broad range of antibiotics and the spores are highly resistant to antibiotics and can withstand many forms of chemical attack, e.g. most high-level disinfectants. The most important problem in treating CDI is the high recurrence rate. Various factors, such as the need to continue treatment with the inciting antibiotic, have been associated with this (see under 'Prognostic criteria and criteria for disease severity'). The antibiotics needed to kill the vegetative bacteria do not kill the spores and might even contribute to recurrence by disrupting the normal gut flora even further. Individuals who suffer a recurrence may enter a repetitive cycle of recurrences, leading to exhaustion and protein-losing enteropathy. A second problem in treating CDI is the fact that in severe forms of CDI antibiotics may fail resulting in progressive colitis with high morbidity and mortality. Several factors may play a role in this, such as a time lag for antibiotics to reach adequate intracolonic levels [1] and possibly the fact that a systemic inflammatory response due to severely damaged colonic mucosa may persist some time after removal of the etiological agent.

Since treatment of CDI can be complicated by these many problems, the CDI Guidance Document Executive Committee decided that there was a need for this evidence-based guideline.

Objective

The objective of this guideline was to evaluate the available evidence concerning treatment of CDI and formulate recommendations for treatment.

Update methodology

Studies on CDI treatment were found with a computerized literature search of PUBMED using the terms "*Clostridium difficile* AND (treatment OR trial)". All randomized and non-randomized trials investigating the effect of an intervention on the clinical outcome (resolution or recurrence of diarrhoea; incidence of complications) of CDI published in any language were included. Studies investigating carriage or other purely microbiological parameters were not considered sufficient evidence for treatment strategies. The resulting literature from 1978 was reviewed and analyzed. Furthermore, systematic reviews from the Cochrane Library and the guideline by the Infectious Diseases Society of America (IDSA) were evaluated. Recommendations were based on a systematic assessment of the quality of evidence. For indicating the quality of evidence and weight of recommendations the system according to The Canadian Task Force on the Periodic Health Examination was used (table 1).

Three draft versions of the guideline were written by three authors (MB, EK, JvD) and criticized by the Executive Committee and advisors. A consensus was reached, resulting in the final version.

Definitions

Criteria for the diagnosis of CDI

Pseudomembranous colitis, which is an endoscopic diagnosis, is caused by *C. difficile* in the vast majority of cases and therefore may suffice for the diagnosis of CDI in the absence of an obvious other cause. In the rest of the cases, a combination of symptoms and signs plus microbiological evidence of toxin-producing *C. difficile* in stool and absence of another cause is necessary. Compatible clinical pictures are diarrhoea, ileus and toxic megacolon. Diarrhoea is defined as loose stools, i.e. taking the shape of the receptacle or corresponding to Bristol stool chart types

Table 1 Strength of recommendation and quality of evidence according to The Canadian Task Force on the Periodic Health Examination

Strength of recommendation:	
A:	good evidence to support a recommendation
B:	moderate evidence to support a recommendation
C:	poor evidence to support a recommendation
Quality of evidence:	
I:	evidence from ≥ 1 properly randomized, controlled trial
II:	evidence from ≥ 1 well-designed clinical trial, without randomization; from cohort or case-controlled analytic studies (preferably from ≥ centre); from multiple time-series; or from dramatic results from uncontrolled experiments
III:	evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees

5 to 7 [2], plus a stool frequency perceived as too high by the patient. Faecal incontinence may be a part of the disease. Ileus in the context of CDI is defined as signs of severely disturbed bowel passage such as vomiting and absence of stool, combined with radiological signs of bowel distension. Toxic megacolon is defined as radiological signs of distension of the colon combined with signs of a severe systemic inflammatory response. We refer to the ESCMID guideline on diagnosis of CDI, which is currently being prepared, for information on microbiological evidence for CDI. The above-mentioned criteria are largely in line with the recommendations by the American Ad Hoc *C. difficile* surveillance working group [3] and the European Study Group for *C. difficile* [4].

Prognostic criteria and criteria for disease severity

Outcome measures of CDI comprise complications, mortality and recurrences. It is difficult to set a rigid set of criteria for the assessment of prognosis and severity of CDI. First, surprisingly little research has been done on clinical predictors of outcome. Second, prognostic markers have not been validated in prognostic studies. Third, prognosis depends on disease severity and other prognostic factors, such as age, comorbidity, admission to an intensive care unit and antiperistaltic and immunosuppressive medication. It is unknown what the weight of these prognostic factors is in comparison to assessed disease severity.

Possible features of severe colitis that have been linked to a higher chance of recurrence are faecal incontinence [5], the endoscopic finding of pseudomembranous colitis [6] and longer cumulative duration of previous episodes of CDI [7]. Leukocytosis

(leukocyte count > 20 · 10^9/l) has been associated with a high mortality rate in CDI [8], a complicated course [9], refractoriness to therapy [6] and risk of recurrence [9]. Hypoalbuminaemia (< 25 g/l) has also been associated with a high mortality rate in CDI [8] and refractoriness to therapy [6,10,11]. However, since it may be seen as a result of malnutrition or protein-losing enteropathy in longstanding disease, as a negative acute phase protein in acute disease, and as a marker for comorbidity (e.g. liver cirrhosis, nephrotic syndrome, wasting) this feature may be too heterogeneous to be a reliable marker for severe disease.

Factors associated with unfavourable outcome that are no direct markers of severe colitis include high age, comorbidity, a decreased antibody response, gastric acid suppressants and need to prolong inciting antibiotic therapy. High age has been associated with a complicated course [12] and recurrence [9,12]. Comorbidity has been associated with a high mortality rate [8] and a higher chance of recurrence [13]. A decreased humoral immune response against Clostridial toxins TcdA and TcdB has been associated with a higher chance of recurrence and longer duration of symptoms [14,15], although other studies did not find this association. Use of H2-antagonists has been associated with a higher chance of recurrence [5] and use of proton pump inhibitors has been associated with refractoriness to therapy [16]. Also the need to continue the inciting antibiotic has been associated with refractoriness to therapy [16]. However, it is unclear whether the use of gastric acid suppressants and the need to continue antibiotics have a causal relationship with unfavourable outcome or whether they are markers of more severe comorbidity. Obviously, admission to an ICU is an unfavourable prognostic feature [6,11].

Markers of severe colitis

Markers that could reasonably be assumed to correlate positively with severity of colitis are mentioned below, although we must stress that the prognostic value of these markers is uncertain. Obviously, markers should not be attributable to a concomitant disease, if they are to be regarded as a marker of severe CDI. Ideally, markers should be obtainable at the earliest time in the disease course to be a predictor of outcome.

Physical examination:
- fever (core body temperature > 38.5 °C)
- rigours (uncontrollable shaking and a feeling of cold followed by a rise in body temperature)
- haemodynamic instability including signs of distributive (vasodilatory; septic) shock
- signs of peritonitis, including decreased bowel sounds, abdominal tenderness, rebound tenderness and guarding
- signs of ileus, including vomiting and absent passage of stool

Admixture of blood with stools is rare in CDI and the correlation with severity of disease is uncertain.

Laboratory investigations:
- marked leukocytosis (leukocyte count > 15 · 10^9/l)
- marked left shift (band neutrophils > 20% of leukocytes)
- rise in serum creatinine (>50% above the baseline)
- elevated serum lactate

Colonoscopy or sigmoidoscopy:
- pseudomembranous colitis

There is insufficient knowledge on the correlation of endoscopic findings compatible with CDI, such as oedema, erythema, friability and ulceration, and the severity of disease.

Imaging:
- distension of large intestine
- colonic wall thickening including low-attenuation mural thickening
- pericolonic fat stranding
- ascites not explained by other causes

The correlation of haustral or mucosal thickening, including thumbprinting, pseudo-polyps and plaques, with severity of disease is unclear.

Prognostic markers other than disease severity
- high age (≥ 65)
- serious comorbidity and ICU admission
- immunodeficiency

Criteria for response, failure and recurrence in the treatment of CDI

Treatment response is present when either stool frequency decreases or stool consistency improves and parameters of disease severity (clinical, laboratory, radiological) improve and no new signs of severe disease develop. In all other cases, there is treatment failure. It is only reasonable to evaluate treatment response after at least three days, assuming that the patient is not worsening on treatment. Treatment with metronidazole, in particular, may only result in a clinical response after three to five days [1,16]. After clinical response, it may take weeks for stool consistency and frequency to become entirely normal [17]. Recurrence is present when after an initial response stool frequency increases for two consecutive days and stools become looser or new signs of severe disease develop and microbiological evidence of toxin-producing *C. difficile* in stool is present without evidence of another cause.

It is impossible to distinguish recurrence due to relapse (renewed symptoms from already present CDI) from recurrence due to reinfection in daily practice.

Overview of medical treatment options available for CDI

There is an increasing body of evidence on treatment of CDI, both initial (tables 2 [6, 18-32], 3 [17, 33-36] and 4 [9,11,13,15,37-48]) and recurrent episodes (tables 5 [33,49-52] and 6 [7,53-68]). Tables 2, 3 and 5 report the evidence from randomized trials with comments on methodology. It is difficult to compare these studies because of differences in diagnostic criteria, exclusion of co-pathogens, severity of CDI, co-morbidity, inciting antibiotics and concomitant use of antibiotics. Moreover, these studies usually have endpoints of clinical cure or microbiological cure. However, the definition of clinical cure and recurrence is highly variable. Patients seldom have normal stools directly after treatment of CDI. With respect to microbiological cure, the significance of persistently or recurrently positive stool toxin tests or cultures is not clear. Furthermore, it is not possible to distinguish relapse from reinfection. Lastly, the number of participants of most trials is small. In conclusion, we need more randomized controlled trials on CDI treatment.

It is important to realize that several experimental treatment options are not widely available, such as toxin-binding resins and polymers and specific immunotherapy.

Stopping the inciting antibiotic without antibiotic treatment

It is unknown what the rate of spontaneous resolution is in patients with mild CDI. In one study [40], spontaneous recovery rate in hospitalized patients with diarrhoea and a positive toxin assay who did not undergo endoscopy or had no pseudomembranous colitis on colonoscopy was 33%. More antibiotics after stopping the inciting antibiotic might increase the chance of subsequent recurrence, since gut flora will be exposed to a second antibiotic with a different spectrum (i.e. metronidazole). It may therefore be prudent to only stop the inciting antibiotic in the case of mild CDI, while closely monitoring the patient.

Oral antibiotics

There is only one placebo-controlled trial investigating the effectiveness of antibiotics for CDI and it had very few participants. Several antibiotics have been compared to each other. Oral administration of the glycopeptides vancomycin and teicoplanin appears most effective in inducing both clinical cure and microbiological cure, especially in severe CDI. The difficulty is how to define severe CDI. In one prospective, randomized, and blinded study [6], which evaluated the efficacy of vancomycin versus metronidazole according to disease severity, the diagnosis of severe CDI was based on age, body temperature, albumin level and leukocyte count. Vancomycin proved to be superior over metronidazole in cases of severe CDI. Two trials investigating the efficacy of the toxin-binding polymer, tolevamer [34,35], also showed superiority of oral vancomycin over metronidazole in severe cases. A recent Cochrane systematic review [70] has examined the available literature on antibiotic treatment options of CDI and concluded that teicoplanin is the most effective antibiotic treatment for moderate to severe CDI and vancomycin has no superiority over metronidazole. However, this review did not include the above-mentioned recent studies. It seems likely that the effectiveness of teicoplanin and vancomycin is in the same range.

Oral metronidazole is also very effective in inducing a response and has the advantage of low cost and the fact that it may contribute less to the emergence of vancomycin-resistant enterococci.

If metronidazole is indeed less effective than glycopeptides, this may be explained by the low levels metronidazole reaches in the colon, since it is absorbed in the small intestine and then excreted again in the bile and in the inflamed colon, whereas glycopeptides are not absorbed. Different doses of oral vancomycin have been used, but only one small randomized trial [22] has compared high versus low dose vancomycin and found no statistically significant difference. Since low doses of oral vancomycin result in high concentrations in stool, there is no need to treat with high doses, except in an attempt to reach sufficient concentrations in the colon when administering vancomycin by nasogastric tube in a patient with ileus. Given the poor faecal concentrations of metronidazole achieved following a 500 mg 8-hourly dose, lower doses (e.g. 250 mg 6-8 hourly) should be less effective. Several studies , however, have used lower doses, usually with good results [6,7,19,27, 28,34,35]. Even a modest increase in the MIC of metronidazole for *C. difficile* might result in insufficient faecal antibiotic concentrations to inhibit (vegetative) bacteria. Metronidazole resistance is to be regarded as exceedingly rare. However, the emergence of reduced susceptibility to metronidazole has recently been reported in UK *C. difficile* strains [1,71,72]. No reduced susceptibility to vancomycin was observed. The exact mechanism of reduced susceptibility to metronidazole remains to be determined. Notably, there is also evidence that inactivation of metronidazole occurs in the presence of gut contents, possibly due to metabolism by enterococci [73].

Oral bacitracin and fusidic acid seem to be less effective than vancomycin and metronidazole, respectively, although this has not convincingly been demonstrated. Currently, there is insufficient evidence to advocate the use of the rifamycin derivative rifaximin, to which resistance has been noted, and the antiprotozoal/ anthelminthic nitazoxanide, which has been shown to be statistically similar to metronidazole in a small prospective randomized trial [28], but whose non-inferiority to vancomycin

Table 2 Randomized controlled trials of antibiotic treatment of initial CDI .Initial cure rate as a percentage of all patients and relapse rate as a percentage of initially cured patients.

Trial	Treatment	Number of patients	Cure [%]	Relapse [%]
Keighley 1978 [18]	vancomycin 125 mg qid, 5 days	9	78	0
	placebo	7	14	-
	No clear case definition. No description of allocation of treatment. Only data of patients with toxin-positive stool shown. Unclear length of follow-up and incidence or relapse in placebo group. $p < 0.02$ for comparison of cure rates.			
Teasley 1983 [19]	vancomycin 500 mg qid, 10 days	32	100	19
	metronidazole 250 mg qid, 10 days	32	97	6
	Only data of patients with toxin-positive stools or pseudomembranous colitis shown. Per-protocol analysis. Follow-up 21 days. Differences not statistically significant.			
Young 1985 [20]	vancomycin 125 mg qid, 7 days	21	86	33
	bacitracin 20000 U qid, 7 days	21	76	42
	Double-blind. 25% drop-out during follow-up of bacitracin group. Follow-up 5 weeks. Differences not statistically significant.			
Dudley 1986 [21]	vancomycin 500 mg qid, 10 days	15	100	20
	bacitracin 25000 U qid, 10 days	15	80	42
	Double-blind. Patients had leukocytosis, fever or abdominal pain. 29% drop-out in vancomycin group, 12% in bacitracin group. Per-protocol analysis. Unclear definition of failure ('worsening during treatment'). Failing patients crossed over to alternate drug. Interruption of study drug in vancomycin group for a mean of 2.8 days and in bacitracin group for a mean of 1.8 days. Unclear length of follow-up. Differences not statistically significant.			
Fekety 1989 [22]	vancomycin 125 mg qid, mean 10.6 days	24	100	21
	vancomycin 500 mg qid, mean 10.1 days	22	100	18
	Variable duration of therapy. 18% dropout rate. Per-protocol analysis. Unclear length of follow-up. Differences not statistically significant.			
Boero 1990 [23]	vancomycin 500 mg bid, 10 days	10	100	-
	rifaximin 200 mg tid, 10 days	10	90	-
	Article in Italian. Patients had diarrhoea, abdominal pain and fever. No description of allocation of treatment. Unclear definition of cure. Differences not statistically significant.			
De Lalla 1992 [24]	vancomycin 500 mg qid, 10 days	20	100	20
	teicoplanin 100 mg bid, 10 days	26	96	8
	No description of allocation of treatment. Per-protocol analysis. Unclear length of follow-up ('at least 1 month'). Differences not statistically significant.			
Wiström 1994 [25]	teicoplanin 100 mg qid, 3 days, followed by 100 mg bid, 4 days	24	96	35
	teicoplanin 100 mg bid, 7 days	23	70	50
	Double-blind. Outcome of 'improvement, but not cure' (2 loose stools per day or 1 loose stool per day with fever or cramps) was counted as failure. 3 patients with improvement in bid group; 1 in qid group. Follow-up 5 weeks. $p = 0.02$ for comparison of cure rates. Relapse rates not statistically different.			
Wenisch 1996 [26]	vancomycin 500 mg tid, 10 days	31	94	17
	metronidazole 500 mg tid, 10 days	31	94	17
	teicoplanin 400 mg bid, 10 days	28	96	7
	fusidic acid 500 mg tid, 10 days	29	93	30
	Follow-up 30 days. Only statistically significant difference was relapse rate of fusidic acid versus teicoplanin ($p = 0.042$).			
Wullt 2004 [27[	metronidazole 400 mg tid, 7 days	55	93	30
	fusidic acid 250 mg tid, 7 days	59	83	30
	Double-blind. 13% drop-out during treatment; 15% further drop-out during follow-up. Per-protocol analysis. Follow-up 35 days. Differences not statistically significant.			
Musher 2006 [28]	metronidazole 250 mg qid, 10 days	34	82	30
	nitazoxanide 500 mg bid, 7 days	40	90	26
	nitazoxanide 500 mg bid, 10 days	36	89	16
	No definition of relapse. Double-blind. 23% drop-out during treatment. Per-protocol analysis. Follow-up 31 days. Differences not statistically significant.			
Lagrotteria 2006 [29]	metronidazole 500 mg tid, 10 days	20	65	38
	metronidazole 500 mg tid + rifampicin 300 mg bid, 10 days	19	63	42
	Intention-to-treat analysis. Follow-up 40 days. Differences not statistically significant.			
Zar 2007 [6]	vancomycin 125 mg qid, 10 days	71	97	7
	metronidazole 250 mg qid, 10 days	79	84	14
	Double-blind. 13% drop-out during treatment. Per-protocol analysis. Follow-up 21 days. $p = 0.006$ for comparison of cure rates. $p = 0.27$ for comparison of relapse rates. The original protocol was stratified in a group with mild and a group with severe disease (based on age, fever, albumin level and leukocyte count), which resulted in a larger difference between cure rates in the group with severe disease and a statistically non-significant difference between cure rates in the group with mild disease.			

Table 2 Continued.

Trial	Treatment	Number of patients	Cure [%]	Relapse [%]
Louie 2009 [30]	fidaxomicin 50 mg bid, 10 days	14	71	8
	fidaxomicin 100 mg bid, 10 days	15	80	0
	fidaxomicin 200 mg bid, 10 days	16	94	6
	Open-label. Patients with signs of highly severe CDI (> 12 bowel movements per day, vomiting, severe abdominal tenderness, ileus, WBC > 30, toxic megacolon) were excluded. Cure = complete resolution of diarrhoea. Follow-up 6 weeks after end of treatment.			
Musher 2009 [31]	vancomycin 125 mg qid, 10 days	27	74	7
	nitazoxanide 500 mg bid, 10 days	22	77	5
	CDI = stool EIA for toxin A or B positive AND (temperature > 38.3 °C OR abdominal pain OR leukocytosis). Patients with > 1 episode in preceding 6 months. 12% dropout rate during treatment. Double-blind, placebo-controlled. Modified intention-to-treat analysis. Industry-sponsored. Cure = complete resolution of symptoms during 3 days after completion of therapy. Per-protocol analysis: 87 vs. 94% cure. Follow-up 31 days after start of treatment. No differences in severity subgroups. Differences not statistically significant.			
Louie 2009 [32]	vancomycin 125 mg qid , 10 days	284	90	24
	fidaxomicin 200 mg bid, 10 days	265	92	13
	Unpublished trial			

Table 3 Randomized controlled trials of non-antibiotic treatment of initial CDI. Initial cure rate as a percentage of all patients and relapse rate as a percentage of initially cured patients.

Trial	Treatment	Number of patients	Cure [%]	Relapse [%]
Probiotics:				
McFarland 1994 [33]	vancomycin or metronidazole + *Saccharomyces boulardii* $2{\cdot}10^{10}$ CFU/ day, 4 weeks	31	-	19
	vancomycin or metronidazole + placebo	33	-	24
	Double-blind. No control for type, duration or dose of antibiotic. Unclear definition of relapse. Follow-up 8 weeks after start of treatment. p = 0.86 for comparison of relapse rates.			
Toxin-binding resins and polymers:				
Louie 2006 [17]	tolevamer 1 g tid, 14 days + placebo	94	60	16
	tolevamer 2 g tid, 14 days + placebo	91	79	7
	vancomycin 125 mg qid, 10 days + placebo	94	91	19
	Non-inferiority trial. Patients with stool frequency > 12 per day or abdominal pain were excluded. Tolevamer could be prolonged when inciting antibiotic could not be stopped. Double-blind. 23% drop-out. Per-protocol analysis. Cure rate of tolevamer 2g non-inferior in comparison with vancomycin (Chow-test p = 0.03). Non-inferiority of tolevamer 1g compared with vancomycin could not be demonstrated. p = 0.05 for comparison of relapse rates of tolevamer 2g with vancomycin. Relapse rates of tolevamer 1g and vancomycin not statistically different. Follow-up 6 – 8 weeks.			
Louie 2007 [34]	tolevamer 3g tid, 14 days	266	47	3
	vancomycin 125 mg qid, 10 days	134	81	23
	metronidazole 375 mg qid, 10 days	143	72	27
	Unpublished trial.			
Bouza 2008 [35]	tolevamer 3g tid, 14 days	268	42	6
	vancomycin 125 mg qid, 10 days	125	81	18
	metronidazole 375 mg qid, 10 days	135	73	19
	Unpublished trial.			
Immunotherapy:				
Lowy 2009 [36]	MDX-066 and MDX-1388 (intravenously administered monoclonal antibodies against TcdA and TcdB) after standard antimicrobial therapy	101	-	7
	placebo after standard antimicrobial therapy	99	-	25
	Unpublished trial. Follow-up 12 weeks.			

Table 4 Observational studies for treatment of initial CDI.

Trial	Treatment	Number of patients	Cure [%]	Relapse [%]
Antibiotics:				
Bartlett 1980 [37]	vancomycin	79	96	14
Silva 1981 [38]	vancomycin	16	100	13
Cherry 1983 [39]	metronidazole	13	100	15
Bartlett 1984 [40]	vancomycin	189	97	24
de Lalla 1989 [41]	vancomycin 500 mg qid, 10 days	23	100	13
	teicoplanin 200 mg bid, 10 days	22	100	0
Olson 1994 [42]	metronidazole	632	98	6
	vancomycin	122	99	10
Kyne 2001 [15]	metronidazole	44	?	50
Fernandez 2004 [11]	metronidazole	99	62	?
Musher 2005 [43]	metronidazole	207	78	28
Pépin 2005 [9]	metronidazole	1123	84	29
	vancomycin	112	?	28
Louie 2005 [44]	difimicin varying dose	45	91	5
Musher 2007 [45]	nitazoxanide 500 mg bid, 10 days Patients first failed metronidazole.	35	74	27
Al Nassir 2008 [16]	metronidazole** Ten patients switched to vancomycin.	34	>90	12
	vancomcyin	18	>90	11
Herpers 2009 [46]	tigecycline varying duration Severe CDI. Follow-up at least 3 months.	4	100	0
Toxin-binding resins and polymers:				
Mogg 1982 [47]	colestipol 10 g qid, 5 days Originally set up as a randomized placebo-controlled trial. Placebo group was merged with historical control, however. Only 6 patients had toxin-positive stool..	12	25	-
Passive immunotherapy with immune whey:				
van Dissel 2001 [48]	metronidazole or vancomycin followed by immune whey protein concentrate, 14 days 56% of patients had recurrent CDI; mean follow-up 333 days.	16	100	0
Numan 2007 [13]	metronidazole or vancomycin followed by immune whey protein concentrate, 14 days 109 episodes; 101 patients; 40% of patients had recurrent CDI.	109	100	10

Table 5 Randomized controlled studies of treatment of recurrent CDI.

Trial	Treatment	Number of patients	Failure* [%]
Probiotics:			
McFarland 1994 [33]	vancomycin or metronidazole + *Saccharomyces boulardii* $2{\cdot}10^{10}$ CFU/ day, 4 weeks	26	35
	vancomycin or metronidazole + placebo	34	65
	Double-blind. No control for type, duration or dose of antibiotic. Unclear definition of relapse. Follow-up 8 weeks after start of treatment. p = 0.04 for comparison of failure rates.		
Surawicz 2000 [49]	vancomycin 500 mg qid, 10 days, followed by *Saccharomyces boulardii* $2{\cdot}10^{10}$ CFU/ day, 4 weeks	18	17
	vancomycin 500 mg qid, 10 days, followed by placebo	14	50
	vancomycin 125 mg qid, 10 days, followed by *Saccharomyces boulardii* $2{\cdot}10^{10}$ CFU/ day, 4 weeks	45	51
	vancomycin 125 mg qid, 10 days, followed by placebo	38	45

Table 5 Continued.

Trial	Treatment	Number of patients	Failure* [%]
	metronidazole 1g/day, 10 days, followed by *Saccharomyces boulardii* $2{\cdot}10^{10}$ CFU/ day, 4 weeks	27	48
	metronidazole 1g/ day, 10 days, followed by placebo	26	50
	Follow-up 5 months after completion of study drug. p = 0.05 for the comparison of failure rates in patients who received 500 mg of vancomycin qid. Drop-out in this group was 22%. No further statistically significant differences.		
Wullt 2003 [50]	metronidazole 400 mg tid, 10 days + *Lactobacillus plantarum* 299v $5{\cdot}10^{10}$ CFU/ day, 38 days	12	42
	metronidazole 400 mg tid, 10 days + placebo	9	67
	Double-blind. 28% drop-out. Follow-up 70 days. Difference not statistically significant..		
Lawrence 2005 [51]	vancomycin or metronidazole followed by *Lactobacillus* GG $6{\cdot}10^{11}$ CFU/ day, 21 days	8	38
	vancomycin or metronidazole followed by placebo	7	14
	Patients blinded. No control for type, duration or dose of antibiotic. Follow-up 60 days after completion of antibiotic. Difference not statistically significant.		
Passive immunotherapy with immune whey:			
Mattila 2008 [52]	colostral immune whey 200 ml tid + placebo, 14 days	18	44
	metronidazole 400 mg tid + placebo, 14 days	20	45
	Double-blind. Multi-centre trial. Follow-up 70 days. Difference not statistically significant.		

* Non-response or relapse

Table 6 Observational studies for treatment of recurrent CDI.

Trial	Treatment	Number of patients	Failure* [%]	Mean follow-up
Antibiotics:				
Buggy 1987 [53]	vancomycin 125 mg qid + rifampicin 600 mg bid, 7 days	7	0	12 m
McFarland 2002 [7]	vancomycin 1 – 2 g/day	14	71	59 d
	vancomycin <1 g/day	48	54	59 d
	vancomycin ≥2 g/day	21	43	59 d
	vancomycin taper	29	31	80 d
	vancomycin pulse	7	14	80 d
	metronidazole <1 g/day	29	45	59 d
	metronidazole 1.5 g/day	5	40	59 d
	metronidazole 2 g/day	2	0	59 d
Johnson 2007 [54]	vancomycin, 14 days, followed by rifaximin varying dose, 14 days	8	13	233 d
Garey 2008 [55]	rifaximin 400 mg tid, 14 days, followed by rifaximin 200 mg tid, 14 days	5	0	310 d
	rifaximin 400 mg tid, 36 days	1	100	-
Probiotics:				
Gorbach 1987 [56]	metronidazole or bacitracin, 10 days, followed by *Lactobacillus* GG 10^{10} CFU/day, 7–10 days	5	20	-
Biller 1995 [57]	*Lactobacillus* GG $6{\cdot}10^{8}$ CFU/day, 14 days	4	0	11 m
Faecal or bacterial instillation:				
Bowden 1981 [58]	faecal enema	16	19	-
Tvede 1989 [59]	faecal or bacterial enema	6	0	-
Lund-Tønnesen 1998 [60]	faecal instillation through coloscope or gastrostoma	18	17	-

Table 6 Continued.

Trial	Treatment	Number of patients	Failure* [%]	Mean follow-up
Aas 2003 [61]	faecal instillation through nasogastric tube, median 3 courses	16	6	90 d
Jorup-Rönström 2006 [62]	faecal enema	5	0	-
Nieuwdorp 2008 [63]	vancomycin 500 mg qid, followed by faecal instillation by nasoduodenal tube or colonoscopy	7	29	150 d
Borody§	faecal enema	61	10	-
Lund-Tønnesen §	faecal instillation through nasojejunal tube	20	17	-
Moore§	faecal enema	65	3	-
Aas§	faecal instillation through nasogastric tube	9	0	-
Macconnachie 2009 [64]	faecal instillation through nasogastric tube	15	27	-
Immunotherapy:				
Leung 1991 [65]	iv gammaglobulin 400 mg/kg every 3 weeks, 4 – 6 months	5	0	5 m
Beales 2002 [66]	iv gammaglobulin 400 mg/kg day 1 and 21	4	0	7.5 m
	iv gammaglobulin, varying dose	5	40	2.8 m
Wilcox 2004 [67]	iv gammaglobulin 300 to 500 mg/kg, 1 to 6 doses	5	40	86 d
McPherson 2006 [68]	iv gammaglobulin 150 to 400 mg/kg	14	71	6.6 m

* Non-response or relapse
§ As reported by Bakken [69]
d = days; m = months

could not be shown in another trial due to lack of power [31]. As yet, there is also insufficient evidence for routine use of fidaxomicin (OPT-80), an inhibitor of RNA polymerase of gram-positive bacteria although preliminary results of a recently presented study are very promising[32].

Duration of antibiotic therapy

The duration of antibiotics has been ten days in most studies. Occasionally, shorter duration (e.g. seven days) has been studied. We feel that there is insufficient evidence for a shorter duration of therapy with any antibiotic to consider shorter regimens a treatment option.

There is no definitive evidence that taper or pulse regimens with vancomycin are effective in reducing the incidence of relapses. This strategy is mainly based on favourable experience and the theoretical rationale that spores can still germinate long after the clinical symptoms have resolved. McFarland et al. [7] retrospectively compared a standard course of antibiotics, vancomycin taper strategies (gradually decreasing the daily dose of vancomycin with 125 to 750 mg per day from varying starting doses) and vancomycin pulse strategies (125 to 500 mg of vancomycin every 2 to 3 days during a period of usually 3 weeks). They found the recurrence rate to be lowest in pulse regimens (14%), followed by taper regimens (31%) and the standard regimen of vancomycin (54%; average for all dose groups). No other studies investigating taper or pulse regimens have been published. Further studies are needed.

Probiotics

Probiotics may be of value when added to antibiotics, but the studies that have investigated this suffer from major drawbacks such as small numbers, non-randomized allocation of antibiotics to which the probiotics were added and lack of homogeneity between study groups. This is also the conclusion reached by a recent Cochrane systematic review [74]. Therefore, there is insufficient evidence to recommend the addition of probiotics to antibiotics. In addition, several reports of invasive disease have been reported resulting from the use of probiotics such as *Saccharomyces boulardii* in debilitated or immunocompromised patients [75, 76]. Moreover, probiotics were associated with increased mortality, partly due to nonocclusive mesenterial ischemia, in a randomized controlled trial in acute pancreatitis [77].

Treatment when oral administration is not possible

The only parenteral antibiotic therapy for CDI, supported by case series, is metronidazole [78]. Furthermore, several case reports regarding the use of intravenous immunoglobulin have been published but the data do not provide sufficient evidence to support its use. Thus, it is unknown how to best treat patients

with ileus due to CDI. There are some anecdotal reports on delivery of vancomycin to the gut by other means than orally, mainly through intracolonic delivery. Questions regarding the efficacy, optimal dosing and duration of treatment with intracolonic vancomycin are unanswered. The introduction of faecal collector drainage systems has facilitated the use of glycopeptide retention enemas in ICUs, but they are very expensive. Tigecycline appeared useful as salvage therapy as reported in a recent case series of patients with severe CDI complicated by ileus, but these promising findings require confirmation in prospective clinical trials [46]. Faecal transplantation has been performed through instillation with a colonoscope or enemas, but there is insufficient evidence to recommend this.

There are no prospective studies assessing which CDI patients benefit from surgical intervention. One study found that colectomy was most successful in a relatively early stage of the disease, i.e. before lactate exceeds 5.0 mmol/l [80].

Recommendations for the treatment of CDI

Recommendations for medical treatment of initial CDI

In the case of mild CDI (stool frequency < 4 times daily; no signs of severe colitis), clearly induced by the use of antibiotics, it is acceptable to stop the inducing antibiotic and observe the clinical response, but patients must be followed very closely for any signs of clinical deterioration and placed on therapy immediately if this occurs. Theoretic rationale, anecdotic evidence and one case-control study suggest that antiperistaltic and opiate agents should be avoided, especially in the acute setting [81]. There is no evidence that switching to 'low-risk' antibiotics when the antibiotic treatment that triggered the episode of CDI cannot be stopped or its spectrum be narrowed, is effective. It seems rational, however, to always strive to use antibiotics covering a spectrum no broader than necessary. When the inciting antibiotic cannot be stopped, antibiotic treatment for CDI should be initiated. Furthermore, there is no proof that stopping gastric acid suppressants is effective, either.

In all other cases than mild CDI medical treatment for CDI should be started. Antibiotics may be started while awaiting diagnostics when there is sufficient clinical suspicion. We recommend treatment of an initial episode of CDI with the following antibiotics, according to disease severity (implementation category between brackets), when oral therapy is possible:

- non-severe: metronidazole 500 mg tid orally for 10 days (A-I)
- severe: vancomycin 125 mg qid* orally for 10 days (A-I).

* Oral vancomycin may be replaced by teicoplanin 100 mg bid, if available.

CDI is judged to be severe when one or more of the markers of severe colitis mentioned under 'definitions' is present. It is unclear whether moderate disease in a patient with other unfavourable prognostic factors, such as high age and comorbidity, should be regarded as severe. This is left to the judgment of the treating physician. There is no evidence that various genotypes of *C. difficile* should be treated differently if disease severity does not differ.

When oral therapy is impossible, we recommend the following antibiotics, according to disease severity (implementation category between brackets):

- non-severe: metronidazole 500 mg tid intravenously for 10 days (A-III)
- severe: metronidazole 500 mg tid intravenously for 10 days + (A-III)
  intracolonic vancomycin 500 mg in 100 ml of (C-III)
  normal saline every 4 – 12 h
  and/or vancomycin 500 mg qid by nasogastric tube (C-III)

Recommendations for surgical treatment of CDI

Colectomy should be performed to treat CDI in any of the following situations:

- perforation of the colon
- systemic inflammation and deteriorating clinical condition not responding to antibiotic therapy; this includes the clinical diagnoses of toxic megacolon and severe ileus.

Since mortality from colectomy in patients with advanced disease is high, it is recommended to operate in a less severe stage. No definite recommendations on the timing of colectomy can be given. Serum lactate may, inter alia, serve as a marker for severity, where one should attempt to operate before the threshold of 5.0 mmol/l [80].

Recommendations for medical treatment of recurrent CDI

Observational data [12] suggest that the incidence of a second recurrence after treatment of a first recurrence with oral metronidazole or vancomycin is similar. Therefore, we recommend treating a first recurrence of CDI as a first episode, unless disease has progressed from non-severe to severe.

We recommend treatment of recurrent CDI with the following antibiotics (implementation category between brackets):

First recurrence:
See *Recommendations for medical treatment of initial CDI.*

Second recurrence and subsequent recurrences:
If oral therapy is possible:

- vancomycin 125 mg qid* orally for at least 10 days (B-II)
  consider a taper/ pulse strategy (B-II)

* Oral vancomycin may be replaced by teicoplanin 100 mg bid, if available.

If oral therapy is impossible:

- metronidazole 500 mg tid intravenously for 10 – 14 days + (A-III)
  retention enema of vancomycin 500 mg in 100 ml of (C-III)
  normal saline every 4 – 12 h
  and/or vancomycin 500 mg qid by nasogastric tube (C-III)

Recommendation for prophylaxis of CDI

Currently, there is no evidence that medical prophylaxis for CDI is efficacious and therefore we do not recommend prophylactic antibiotics. Of course, other preventive measures should be taken, such as hand hygiene of hospital personnel, prompt isolation of patients suspected of having CDI and prudent use of antibiotics [82].

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Chapter 9

European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for *Clostridium difficile* infection

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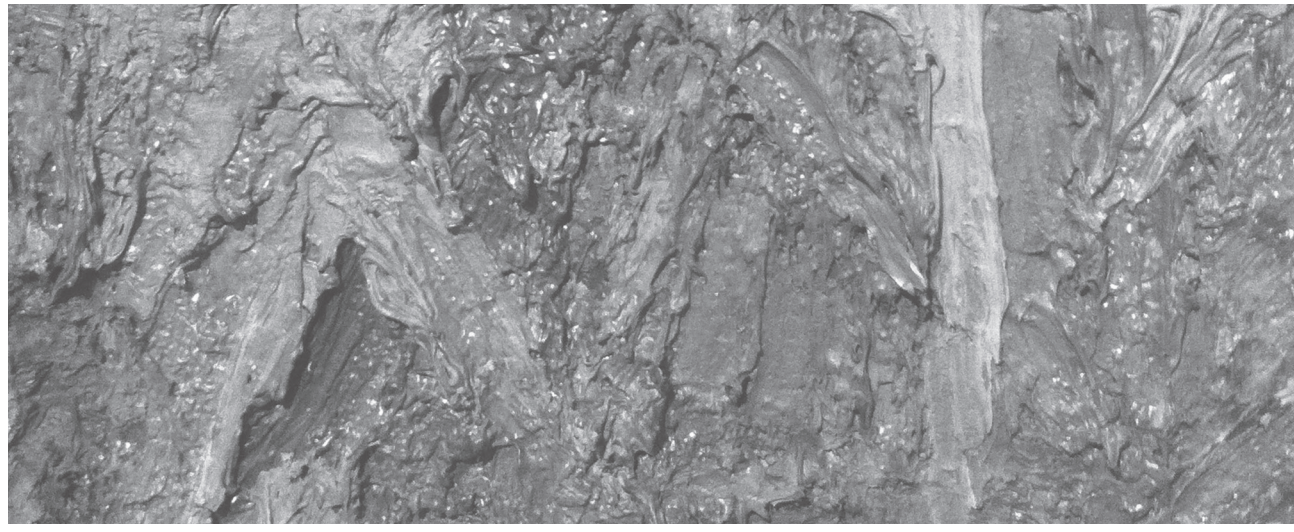

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Abstract

In 2009 the first European Society of Clinical Microbiology and Infection (ESCMID) treatment guidance document for *Clostridium difficile* infection (CDI) was published. The guideline has been applied widely in clinical practice. In this document an update and review on the comparative effectiveness of the currently available treatment modalities of CDI is given, thereby providing evidence-based recommendations on this issue. A computerized literature search was carried out to investigate randomized and non-randomized trials investigating the effect of an intervention on the clinical outcome of CDI. The Grades of Recommendation Assessment, Development and Evaluation (GRADE) system was used to grade the strength of our recommendations and the quality of the evidence. The ESCMID and an international team of experts from 11 European countries supported the process. To improve clinical guidance in the treatment of CDI, recommendations are specified for various patient groups, e.g. initial non-severe disease, severe CDI, first recurrence or risk for recurrent disease, multiple recurrences and treatment of CDI when oral administration is not possible. Treatment options that are reviewed include: antibiotics, toxin-binding resins and polymers, immunotherapy, probiotics, and faecal or bacterial intestinal transplantation. Except for very mild CDI that is clearly induced by antibiotic usage antibiotic treatment is advised. The main antibiotics that are recommended are metronidazole, vancomycin and fidaxomicin. Faecal transplantation is strongly recommended for multiple recurrent CDI. In case of perforation of the colon and/or systemic inflammation and deteriorating clinical condition despite antibiotic therapy, total abdominal colectomy or diverting loop ileostomy combined with colonic lavage is recommended.

Introduction

The previous European Society of Clinical Microbiology and Infection (ESCMID) guidance document, which has been applied widely in clinical practice, dates from 2009 [1]. Meanwhile, new treatments for *Clostridium difficile* infection (CDI) have been developed and limitations of the currently recommended treatment options of CDI are considered. As the current ESCMID treatment guidance document is already implemented in clinical practice, an update of this widely applied guidance document is essential to further improve uniformity of national hospital infection treatment policies for CDI in Europe. In particular, after the recent development of new alternative drugs for the treatment of CDI (e.g. fidaxomicin) in the USA and Europe, there has been an increasing need for an update on the comparative effectiveness of the currently available antibiotic agents in the treatment of CDI, thereby providing evidence-based recommendations on this issue.

The objectives of this document are to:

1. Provide an overview of currently available CDI treatment options
2. Develop an evidence-based update of treatment recommendations

Update methodology

Studies on CDI treatment were found with a computerized literature search of PUBMED and Google Scholar using the terms '*Clostridium difficile* AND (treatment OR trial)'. All randomized and non-randomized trials investigating the effect of an intervention on the clinical outcome (resolution or recurrence of diarrhoea; incidence of complications) of CDI published in any language were included. Studies investigating carriage or other purely microbiological parameters were not considered sufficient evidence for treatment strategies. The resulting literature from 1978 was reviewed and analysed. Furthermore, systematic reviews from the most recent Cochrane analysis [2] and the up-dated guidelines of the Infectious Diseases Society of America, the Australasian Society for Infectious Diseases, the American College of Gastroenterology, and the Health Protection Agency/Public Health England guidance document (http://www.hpa.org.uk) were evaluated [3–5]. Recommendations were based on a systematic assessment of the quality of evidence. The Grades of Recommendation Assessment, Development and Evaluation (GRADE) system was used to grade the strength of our recommendations and the quality of the evidence [6,7].

Draft versions of the guideline were written by the executive committee (consisting of: S. Debast, M. Bauer and E. Kuijper) and criticized by the Executive Committee and advisors. After this, consensus was reached, resulting in the final version. The methods to evaluate the quality of evidence and to reach group consensus recommendations were based on the method described by Ullmann et al. [8].

Definition of the strength of recommendation is given in Table 1. The quality of the published evidence is defined in Table 2a. Grouping quality of evidence into three levels only may lead to diverse types of published evidence being assigned specifically to a level II. To increase transparency in the evaluation of the evidence an index (Table 2b) to the level II recommendations was added where appropriate.

The guideline followed the Appraisal of Guidelines Research and Evaluation Collaboration (AGREE) self-assessment tool [9].

Tabel 1 Definition of the Strength of Recommendation Grade (SoR) ESCMID

Strength	Definition
A	**Strongly** supports a recommendation for use
B	**Moderately** supports a recommendation for use
C	**Marginally** supports a recommendation for use
D	**Supports** a recommendation against use

Definitions

Diagnosis

The diagnosis of CDI is based on (1) a combination of signs and symptoms, confirmed by microbiological evidence of *C. difficile* toxin and toxin-producing *C. difficile* in stools, in the absence of another cause, or (ii) colonoscopic or histopathological findings demonstrating pseudomembranous colitis [1,3,10–12]. There are many different approaches that can be used in the laboratory diagnosis of CDI; however, the best standard laboratory test for diagnosis has not been established. Diagnostic tests for CDI include: (i) detection of *C. difficile* products: cell culture cytoxicity assay (CCA), glutamate dehydrogenase (GDH) and Toxins A and/or B, (ii) toxigenic culture of *C. difficile*, and (iii) nucleic acid amplification tests (NAAT): 16S RNA, toxin genes, GDH genes. Preferably a two- or three-stage algorithm is performed to diagnose CDI, in which a positive first test is confirmed with one or two confirmatory tests or a reference method [3,4,12,13]. Faeces samples could be investigated with an enzyme immunoassay detecting GDH, an enzyme immunoassay detecting toxins A and B, or NAAT detecting Toxin B (TcdB). Samples with a negative test result can be reported as negative. Faeces samples with a positive first test result should be re-tested with a method to detect free faeces toxins, or with a method to detect GDH or toxin genes, dependent on the assay applied as first screening test. If free faeces toxins are absent but *C. difficle* TcdB gene or GDH are present, CDI cannot be differentiated from asymptomatic colonization. Recently, a large study was presented in which several diagnostic

Tabel 2a Definition of the Quality of Evidence (QoE) Level ESCMID. Adapted from ref [8].

Quality of Evidence Level	Definition
I	Evidence from at least 1 properly designed randomized, controlled trial
II	Evidence from at least 1 well-designed clinical trial, without randomization; from cohort or case-controlled analytic studies (preferably from >1centre); from multiple time series; or from dramatic results of uncontrolled experiments
III	Evidence from opinions of respected authorities, based on clinical experience, descriptive case studies, or reports of expert committees

Tabel 2b Definition of the Quality of Evidence (QoE) Index ESCMID. Adapted from ref [8].

Quality of Evidence Index	Definition
r	Meta-analysis or systematic review of randomized controlled trials
t	Transferred evidence i.e. results from different patients‘ cohorts, or similar immune-status situation
h	Comparator group is a historical control
u	Uncontrolled trial
a	Abstract published at an international meeting

algorithms were evaluated to optimize the laboratory diagnosis of CDI [14]. The investigators concluded that two-stage algorithms improve diagnosis of CDI. Two commonly recommended methods in the laboratory diagnosis of CDI are the use of GDH detection in stools as a means of screening for CDI, confirmed by NAAT such as PCR to detect toxigenic strains of *C. difficile* [4,12]. Furthermore, patients with a positive stool toxin had *C. difficile* disease with an increased risk of mortality compared with patients with only a positive toxigenic culture, thereby implying that stool toxin testing should be included in a testing algorithm to optimize *C. difficile* diagnostic testing [15]. Diarrhoea is defined as loose stools, i.e. taking the shape of the receptacle or corresponding to Bristol stool chart types 5– 7, plus a stool frequency of three stools in 24 or fewer consecutive hours or more frequently than is normal for the individual (definition World Health Organization, http://www.who.int/topics/diarrhoea) [1,3,16–18].

Clinical pictures compatible with CDI are summarized in Table 3.

Tabel 3 Clinical pictures compatible with *Clostridium difficile* infection (CDI). Adapted from refs [1,3,11,19,20]

Sign/symptom	Definition
Diarrhoea	Loose stools, i.e. taking the shape of the receptacle or corresponding to Bristol stool chart types 5 to 7 and a stool frequency perceived as too high by the patient
Ileus	Signs of severely disturbed bowel passage such as vomiting and absence of stool and radiological signs of bowel distension
Toxic megacolon	Radiological signs of distension of the colon and signs of a severe systemic inflammatory response

Definition of *Clostridium difficile* infection. An episode of CDI is defined as: A clinical picture compatible with CDI and microbiological evidence of free toxins and the presence of *C. difficile* in stool without reasonable evidence of another cause of diarrhoea. or Pseudomembranous colitis as diagnosed during endoscopy, after colectomy or on autopsy [3,11,19].

Treatment response

Definition of treatment response. Treatment response is present when either stool frequency decreases or stool consistency improves and parameters of disease severity (clinical, laboratory, radiological) improve and no new signs of severe disease develop. In all other cases, treatment is considered a failure. Treatment response should be observed daily and evaluated after at least 3 days, assuming that the patient is not worsening on treatment. Treatment with metronidazole, in particular, may result in a clinical response only after 3–5 days [21–23]. After clinical response, it may take weeks for stool consistency and frequency to become entirely normal [23,24].

Recurrences

Definition of recurrent *Clostridium difficile* infection. Recurrence is present when CDI re-occurs within 8 weeks after the onset of a previous episode, provided the symptoms from the previous episode resolved after completion of initial treatment [4,11].

It is not feasible to distinguish recurrence due to relapse (renewed symptoms from already present CDI) from recurrence due to reinfection in daily practice [20,25–28].

Severity of disease

Definition of severe *Clostridium difficile* infection. Severe CDI is defined as an episode of CDI with (one or more specific signs and symptoms of) severe colitis or a complicated course of disease, with significant systemic toxin effects and shock, resulting in need for ICU admission, colectomy or death [1,4,29].

Clostridium difficile infection without signs of severe colitis in patients with greater age (≥65 years), serious comorbidity, Intensive Care Unit (ICU) admission, or immunodeficiency may also be considered at increased risk of severe CDI [30,31].

An overview of characteristics in patients with CDI that are assumed to correlate with the severity of colitis is given in Table 4 [32–39]. We must stress that the prognostic value of these markers is uncertain.

Tabel 4 Clinical signs and symptoms that could reasonably be assumed to correlate positively with severity of colitis or a complicated course of disease in the absence of another explanation for these findings

Category	Signs/symptoms
Physical examination	- Fever (core body temperature > 38.5 °C) - Rigours (uncontrollable shaking and a feeling of cold followed by a rise in body temperature) - Haemodynamic instability including signs of distributive (vasodilatory; septic) shock - Signs of peritonitis, including decreased bowel sounds, abdominal tenderness, rebound tenderness and guarding - Signs of ileus, including vomiting and absent passage of stool Admixture of blood with stools is rare in CDI and the correlation with severity of disease is uncertain.
Laboratory investigations	- Marked leucocytosis (leukocyte count > $15 \cdot 10^9$/l) - Marked left shift (band neutrophils > 20% of leukocytes) - Rise in serum creatinine (>50% above the baseline) - Elevated serum lactate - Markedly reduced serum albumin (< 30 g/l)
Colonoscopy or sigmoidoscopy	- Pseudomembranous colitis There is insufficient knowledge on the correlation of endoscopic findings compatible with CDI, such as oedema, erythema, friability and ulceration, and the severity of disease.
Imaging	- Distension of large intestine - Colonic wall thickening including low-attenuation mural thickening - Pericolonic fat stranding - Ascites not explained by other causes The correlation of haustral or mucosal thickening, including thumbprinting, pseudopolyps and plaques, with severity of disease is unclear.
Other	- High age (≥ 65) - Serious comorbidity and/or immunodeficiency - ICU admission

Clinical prediction markers

Evidence. Clinical studies indicate superiority of specific treatment strategies depending on the severity of disease. In addition, alternative treatment options have been developed, that may be more effective in preventing recurrence of disease. Unfortunately some of the novel treatment strategies can be very expensive, and may only be cost-effective for a certain group of patients depending on the stage and severity of disease. This emphasizes the importance for better identification of clinical markers, preferably early in the course of disease, which might predict the benefit from specific treatment regimens to decrease CDI-related complications, mortality or recurrences. Surprisingly little prospective and validated research has been performed on clinical predictors of outcome [40]. Furthermore, for some complications of CDI, such as ICU admission or death, it is difficult to determine to what extent the complication can be attributed to CDI as opposed to the presenting acute illness(es) or comorbidities.

A wide variety of risk factors for severe or recurrent CDI have been suggested in literature, which makes it difficult to set a rigid clinical prediction rule [1,25,41–46]. Recently, a systematic review was performed to derive and validate clinical rules to predict recurrences, complications and mortality [46]. Most studies were found to have a high risk of bias because of small sample sizes and much heterogeneity in the variables used, except for leucocytosis, serum albumin and age [46]. Bauer et al. used a database of two randomized controlled trials, which contained information for a large patient group (1105 patients) with CDI, to investigate the prognostic value of three markers for severe CDI. They found that both leucocytosis and renal failure are useful predictors of a complicated course of CDI, if measured on the day of diagnosis [45].

A recent meta-analysis of two pivotal randomized controlled trials comparing fidaxomicin and vancomycin revealed previous vancomycin or metronidazole treatment in the 24 h before randomization, low eosinophil count (<0.1 9 109/L) and low albumin level to be independent predictors of persistent diarrhoea or death in the first 12 days [40]. Recently Miller et al. [36] analysed the same two clinical therapeutic trials to derive and validate a categorization system to discriminate among CDI patients and correlate the grouping with treatment response. They concluded that a combination of five clinical and laboratory variables measured at the time of CDI diagnosis, combined into a scoring system, were able to accurately predict treatment response to CDI therapy with fidaxomicin and vancomycin. These variables include: age, treatment with systemic antibiotics, leucocyte count, albumin and temperature (ATLAS).

C. Strain type has been suggested as an additional cause of excess morbidity, disease severity and higher recurrence rates of CDI. In a Canadian study [47], PCR ribotype 027 was correlated with more severe disease and fatal outcome among

patients at almost all ages. Some studies on the other hand suggested that PCR ribotype 027 strains might only be associated with worse outcome in settings where 027 strains are epidemic, and not in an endemic situation [38,48]. However, these findings are questioned by others [49]. Recently, a large study by Walker et al. clearly showed that strain types varied in the overall impact on mortality and biomarkers (predominantly those associated with inflammatory pathways) [50]. Besides *C. difficile* PCR ribotype 027, other strains are also associated with outbreaks and severe *C. difficile* infection, e.g. PCR ribotype 078 [51]. Despite increased virulence of specific strain types, the value of the PCR ribotype as a prediction marker for disease severity may be limited, as the ribotype involved in an infection is commonly not known upon diagnosis. However, in an epidemic situation the PCR ribotype may be taken into account in deciding on the choice of empirical treatment regimens [21,39].

The level of host immune response to *C. difficile* exposure has been shown to be an important determinant of the severity and duration of clinical manifestations [52–57]. Anti-toxin antibody levels have been demonstrated to be higher in healthy adult controls compared with healthy children, and levels were found to fall with increasing age. In addition, anti-toxin antibodies increased after resolution of diarrhoea, which coincided with decreased incidence of CDI recurrence [57]. Inability to mount an adequate humoral immune response (e.g. during use of rituximab) may therefore be an important additional prediction marker for severe and/or recurrent CDI [25,57–62]. Unfortunately, in most cases this information is not available at presentation/diagnosis; also, as the strength of evidence for immunodeficiency as an independent predictor for severe and/ or recurrent CDI is still limited, we did not include this risk factor as a separate prediction marker.

The results from individual studies, reviews and meta-analyses on prognostic markers for CDI were evaluated to reach a group consensus on a selection of markers that may be useful in clinical practice to distinguish patients with increased risk for severe or life-threatening CDI and recurrences. For detailed recommendations we refer to Tables 5 and 6.

Recommendations. *Clostridium difficile* infection is judged to be severe when one or more of the clinical markers of severe colitis mentioned in Table 4 is present, and/or when one or more unfavourable prognostic factors (Table 5) is present:

1. Marked leucocytosis (leucocyte count >15 9 109/L)
2. Decreased blood albumin (<30 g/L)
3. Rise in serum creatinine level (≥133 lM or ≥1.5 times the premorbid level)

Clostridium difficile infection without signs of severe colitis in older patients (≥65 years), serious comorbidity, ICU admission, or immunodeficiency may also be regarded as increased risks of developing severe CDI.

Table 5 Consensus recommendation: prediction markers for severe *Clostridium difficile* infection (CDI) and/or complicated course of disease

Marker	SoR	QoE	Reference(s)	Comment(s)
Age (≥ 65 years)	A	IIr	[32,41,46]	Large cohort study on CDI mortality at 30 d, and review of studies of factors associated with CDI outcome. [41] Systematic review of studies describing the derivation or validation of Clinical Prediction Rules for unfavorable outcomes of CDI [46]
Marked leukocytosis (leukocyte count > $15 \cdot 10^9$/l)	A	IIrht	[32,37,39,45,46,63,64]	Systematic review [46] Cohort study: severity score on malignancy, white blood cell count, blood albumin, and creatinine [37] Retrospective cohort study on risk factors for severe CDI: death < 30d, ICU, colectomy or intestinal perforation. [32]
Decreased blood albumin (< 3.0 mg/dL)	A	IIr	[32,37,40,46,65]	Systematic review [46]
Rise in serum creatinin (>50% above the baseline)	B	IIht	[32,37,41,45]	Depending on the timing of measurement around CDI diagnosis [45]
Comorbidity (severe underlying disease and/or immunodeficiency)	B	IIht	[37,41,63,66]	Comorbidity: wide variety of risk factors described/investigated. Cancer, cognitive impairment, cardiovascular, respiratory and kidney disease. [41] Chronic pulmonary disease, chronic renal disease and diabetes mellitus. [66] History of malignancy.[47] Prior operative therapy, inflammatory bowel disease and intravenous immunoglobulin treatment [36]

Table 6 Consensus recommendation: prediction markers for recurrent *Clostridium difficile* infection (CDI)

Marker	SoR	QoE	Reference(s) *Not complete*	Comment(s)
Age (> 65 years)	A	IIrh	[42,43,46,67]	Meta-analysis: [43] Systematic review: [46] Prospective validation study of risk factor: [42]
Continued use of (non-CDI) antibiotics after diagnosis of CDI and/or after CDI treatment	A	IIrh	[42,43]	Meta-analysis: [43] Prospective validation study of risk factor: [42]
Comorbidity (severe underlying disease) and/or renal failure	A	IIh	[42,45,68]	Prospective validation study of risk factor: comorbidity conditions rated by Horn's index (underlying disease severity) [42]
A history of previous CDI (> 1 recurrences)	A	IIt	[26,40,69–71]	Data from randomized controlled trials: [26,70] Meta-analysis of pivotal randomized controlled trials [40].
Concomitant use of antacid medications (PPI)	B	IIrh	[43,72]	Meta-analyses on recurrent CDI:[43], Meta-analysis on CDI: [72]
Initial disease severity	B	IIth	[42,67]	Prospective validation study of risk factor [42] Longterm population based cohort study [67]

Treatment of *Clostridium difficile* infection

Once CDI is diagnosed in a patient, immediate implementation of appropriate infection control measures is mandatory to prevent further spread within the hospital. These include early diagnosis of CDI, surveillance, education of staff, appropriate use of isolation precautions, hand hygiene, protective clothing, environmental cleaning and cleaning of medical equipment, good antibiotic stewardship, and specific measures during outbreaks. Measures for the prevention and control of CDI ('bundle approach') have been described in an ESCMID guideline by Vonberg et al. [73].
Additional treatment measures include [1,3,4,72,74]:

- Discontinuation of unnecessary antimicrobial therapy
- Adequate replacement of fluid and electrolytes
- Avoidance of anti-motility medications
- Reviewing proton pump inhibitor use

In general it is difficult to compare studies on the treatment of CDI because of the use of variable diagnostic criteria, patient selection and subgroup definitions, stringency of searches for potential enteropathogens, severity of CDI, comorbidities, exposures to causative or concomitant antibiotics, and follow up. Moreover, studies have employed different definitions of clinical and/or microbiological cure and recurrence [2,75]. The variability in definitions and criteria of randomized controlled trials of antibiotic therapy for CDI is illustrated in Table 7. In 13/17 randomized controlled trials of antibiotic treatment of initial CDI, recurrences and duration of follow up were defined. Follow up varied from 3 to 6 weeks after treatment for CDI. In 6/17 randomized controlled trials definitions for severity of disease were given. In most of the studies very severe and/or life-threatening CDI was excluded.

A Cochrane analysis published in 2011 reviewed 15 studies on the antibiotic treatment for CDI in adults [2]. The risk of bias was rated high in 12 of the 15 included studies. The authors concluded that a specific recommendation for the antibiotic treatment of CDI could not be made. Nevertheless, and in spite of the observed limitations, it is apparent that a clear and up-to-date guideline on the treatment of CDI is urgently needed for clinical practice. For this purpose the strength of a recommendation and the quality of evidence are assigned in two separate evaluations in this guideline, hence allowing an assessment of the strength of a recommendation independent of the level of supportive evidence (Tables 1 and 2).
To improve clinical guidance in the treatment of CDI, treatment recommendations are specified for various patient groups:

A. Initial CDI: non-severe disease
B. Severe CDI

C. C: First recurrence or (risk of) recurrent CDI
D. D: Multiple recurrent CDI
E. Treatment of CDI when oral administration is not possible

The following treatment options are considered:

1. Oral and non-oral antibiotics
2. Toxin-binding resins and polymers
3. Immunotherapy
4. Probiotics

Faecal or bacterial intestinal transplantation

A. Initial *Clostridium difficile* infection: non-severe disease

Oral antibiotic therapy for non-severe disease

Evidence. The antibiotics commonly used to treat CDI are oral metronidazole or oral vancomycin.

Oral metronidazole has been shown to be effective in inducing a clinical response and has the advantage of low cost and is assumed to be associated with reduced vancomycin-resistant enterococci (VRE) selection risk. In a pooled intention-to-treat analysis (treating exclusions, deaths and relapses as treatment failures) of three randomized controlled trials comparing symptomatic cure between metronidazole and vancomycin [77,84,88], no statistically significant differences were found [2,75]. Symptomatic cure was achieved in 79% of patients who received vancomycin compared with 71% of patients who received metronidazole (three studies; 335 patients; RR 0.91; 95% CI 0.81–1.03, p 0.14) [2]. However, a recently presented pooled analysis of data from two phase three randomized controlled trials on the use of tolevamer, comparing resolution of diarrhoea and abdominal pain (clinical success) for vancomycin versus metronidazole, showed that overall metronidazole was inferior to vancomycin [92]. Vancomycin significantly improved clinical success (81.1% vs 72.7%; OR 1.681; 95% CI 1.114–2.537; p 0.0134). In addition a retrospective analysis of case records of hospitalized patients with CDI showed that the symptomatic response time was significantly ($p < 0.01$) shorter in patients treated with vancomycin (3.0 days, $n = 22$) compared with those given metronidazole (4.6 days, $n = 28$) [23]. Oral metronidazole is usually recommended for treatment of non-severe disease, whereas oral vancomycin is generally preferred for treatment of severe infections [1,3–5].

Decreased clinical effectiveness of metronidazole treatment for specific ribotypes causing CDI, e.g. PCR ribotype 027, has been described [93]. Although changes in antibiotic resistance and ribotype prevalence have been reported, in vitro studies

Table 7 Randomized controlled trials of antibiotic treatment of initial *Clostridium difficile* infection (CDI): definitions and criteria of recurrences, follow-up and severity of disease

Trial	Recurrences prior to study	Relapse/recurrences and follow-up	Severity of CDI	Severe CDI excluded/included
[76]	Previous PMC excluded	Recurrences not defined and follow-up not specified	Not defined	Not specified
[77]	Not described	Reappearance of diarrhoea <21 d	Not defined	Not specified
[78]	Not described	Reappearance of diarrhoea <5 wk	Not defined	Not specified
[79]	Not described	Reappearance of diarrhoea after therapy Follow-up: length not clear	Not defined	Not specified
[80]	Not described	"Recurrence of disease": not further specified Follow-up not defined	No definition but judged by physician	Severe/moderate CDI included, mild CDI excluded
[81]	Not described	Not described No follow-up period	Not defined	Not specified
[82]	Not described	Reappearance of diarrhoea and other symptoms ≥1 m Follow-up not further specified	Not defined	Not specified
[83]	Treatment for CDI <6 wk excluded.	Cure followed by return of inclusion criteria CDI <4 wk	Not defined	Not specified
[84]	Not described	Reappearance of diarrhoea and other symptoms <25-30 d	Severity estimated by: number/shape stool, CRP, WBC, ESR	Severe and mild CDI included. Results for PMC specified
[85]	CDI ≤6 m excluded	Reappearance diarrhoea during 28-33 d	Not defined	Not specified. Severe "medical conditions" excluded
[86]	Not specified Excluded oral vanco/metro treatment <7d prior to study (<= 2 doses included)	Reappearance of symptoms < 31 days after start of treatment and after at least 1 negative CD toxin test before retreatment	Not defined	Toxic megacolon excluded
[87]	Previous CDI excluded	Recurrence of diarrhoea during 30 d	Not defined	Not specified. Ileus and toxic megacolon excluded
[88]	Prior failure of treatment for CDI with study-drugs excluded	Recurrence of CD toxin positive diarrhoea within 21 d	Severe CDI defined as severity assessment score ≥ 2 (points). Based on: age (1), Temp (1), Alb (1), WBC (1), endoscopic PMC (2), ICU (2)	Severe and mild CDI included: results specified Life-threatening abdominal complications excluded
[89]	>1 recurrence or relapse within 3 months prior to study excluded	Recurrence of CD toxin positive diarrhoea <6 wk	Severity CDI based on: stools/day, vomiting, ileus, severe abdominal tenderness, WBC, toxic megacolon, life-threatening CDI	Mild to moderately severe CDI included: results not specified Very severe CDI excluded
[90]	>1 recurrence <3 m prior to study excluded Results specified for CDI <90 d before study.	Return of symptoms (toxin positive diarrhoea) <31 d after onset of treatment, or clinical response after empiric re-treatment	Severe CDI defined as severity assessment score ≥ 2 (points). Based on: age (1), stools/day (1), Temp (1), Alb (1), WBC (1)	Severe and mild CDI included: results specified Unstable vital signs or ICU excluded.
[70]	>1 CDI <3 m prior to study excluded. Results specified for patients with/without CDI < 3 m before study.	Reappearance of CD toxin positive diarrhoea <4 wk and need for retreatment for CDI	Mild, moderate and severe CDI: based on bowel movements/day, WBC	Mild, moderate and severe disease included: results specified. Life-threatening or fulminant CDI and toxic megacolon excluded
[91]	>1 CDI <3 m prior to study excluded Results specified for patients with CDI < 3 m before study.	Return of CD toxin positive diarrhoea < 30 d and need for retreatment for CDI	Severe and not-severe CDI based on ESCMID criteria: WBC, Creatinine, Temp.	Severe and not-severe disease included: results specified for severity. Life-threatening or fulminant CDI and toxic megacolon excluded

Alb, serum albumin; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ICU, intensive care unit; PMC, pseudomembranous colitis; WBC, white blood cell count.

indicate that MICs of metronidazole and vancomycin for endemic *C. difficile* have remained relatively low over the years. Brazier et al. concluded that the MICs of metronidazole and vancomycin were not indicative of clinical failure, but MICs for epidemic ribotypes (027, 106 and 001) were several dilutions higher [94]. Indeed there is increasing evidence of the emergence of reduced susceptibility to metronidazole in some *C. difficile* strains, with evidence for clonal spread [95]. Notably, MIC methodology is crucial to the detection of reduced susceptibility to metronidazole; E-tests in particular underestimate the MIC [95,96]. There is also evidence of inferior microbiological efficacy of metronidazole in comparison with vancomycin [21,22]. Although poor gut concentrations of metronidazole alongside reduced susceptibility to metronidazole could explain reduced treatment efficacy, treatment failures have not been associated with decreased susceptibility [95,97,98]. A case–control study found no significant differences in clinical outcome for CDI cases from which strains with reduced susceptibility to metronidazole were recovered versus matched (metronidazole-susceptible) controls [99]. Response to metronidazole was generally poor (slow and prone to recurrence) and the frail elderly patients had a 21% 30-day mortality. However, much larger study groups are needed to determine the clinical significance of CD isolates with reduced susceptibility to metronidazole [99].

Orally administered vancomycin is poorly absorbed from the gastrointestinal tract, and therefore luminal drug levels are high and orders of magnitude are greater than the susceptibility breakpoint concentration for all strains of *C. difficile* tested so far, thereby resulting in a more rapid suppression of *C. difficile* to undetectable levels during therapy and faster resolution of diarrhoea [22,23]. Metronidazole, on the other hand, is well absorbed from the gastrointestinal tract. Mean antibiotic concentrations reported in faeces of patients receiving oral metronidazole range from <0.25 to 9.5 mg/L, and drug concentrations in faeces decrease to undetectable levels as mucosal inflammation improves and diarrhoea resolves [100]. Increased MIC for metronidazole could therefore have implications on clinical cure or recurrences in CDI. Although there are no published reports in which treatment failure has been linked to antimicrobial metronidazole resistance in *C. difficil*e, the pharmacokinetic properties of vancomycin are considered superior to those of metronidazole in severe *C. difficile* disease [88].

There is concern that use of vancomycin may be more likely to promote colonization and transmission of VRE by selection pressure. However, both oral metronidazole and oral vancomycin have been associated with the promotion of persistent overgrowth of VRE in stool samples obtained from colonized patients during CDI treatment, thereby increasing the risk of transmission [101]. In a small study of VRE-colonized patients with CDI, who experienced frequent faecal incontinence, skin and environmental VRE contamination was common during and after resolution of diarrhoea. It was concluded that the frequency of VRE contamination of skin or the environment was similar between patients treated with metronidazole (n = 17) and those given vancomycin (n = 17), although the study clearly had only limited power to examine this issue [102]. In a large retrospective analysis, increased vancomycin use during an outbreak of CDI was not associated with an increase in VRE colonization during a follow-up period of 2 years after the outbreak period [103]. The authors concluded that restriction of vancomycin use during CDI outbreaks because of the fear of increasing VRE colonization might not be warranted. However, the interpretation of the data was complicated by an outbreak of VRE (VanA) cases that was observed after approximately 20 months of increasing preferential use of vancomycin. As the rate of VanA cases subsequently decreased very quickly, the investigators concluded that this temporary increase reflected a localized clonal outbreak unrelated to the CDI therapy at that time [103].

Although vancomycin and metronidazole are effective in the treatment of CDI, they are both broader-spectrum agents that cause significant disruption of the commensal colonic microbiota. A disruption in the commensal microbiota may predispose to recurrent CDI and intestinal colonization by health- care-associated pathogens such as VRE and Candida species. Fidaxomicin appears to cause less disruption of the anaerobic colonization microbiota, and has activity against many VRE strains [104] so it is suggested that the risk of colonization with and transmission of VRE associated with fidaxomicin treatment may be lower compared with vancomycin therapy. A recent study concluded that fidaxomicin was indeed less likely than vancomycin to promote acquisition of VRE and Candida species during CDI treatment. However, selection of pre-existing subpopulations of VRE with elevated fidaxomicin MICs was more common during fidaxomicin therapy [105].

Similar cure rates have been demonstrated for oral vancomycin and oral teicoplanin [82,84]. For bacteriological cure, oral teicoplanin may even be more effective than vancomycin [2,82]. Both glycopeptides are active in vitro against *C. difficile* isolates [106]. Since 2013 teicoplanin does have a licensed indication for CDI and is available for oral administration. Teicoplanin is not available in the USA. For the purpose of this treatment guideline only oral vancomycin is included in the treatment recommendations.

Tables 8 and 9 report the evidence for oral treatment of initial CDI from randomized trials and observational studies with comments on methodology.

Although oral metronidazole absorption is very high and potentially can lead to more systemic side-effects, adverse effects of oral metronidazole are commonly mild to moderate in severity. The most common adverse reactions reported involve the gastrointestinal tract [107]. Rarely, particularly in association with long duration therapy, metronidazole has been linked to more severe safety issues, e.g. peripheral and optic neuropathy [108] and interactions with warfarins [109].

Tabel 8 Randomized controlled trials of oral antibiotic treatment of initial *Clostridium difficile* infection (CDI). Initial cure rate, and sustained response rates as a percentage of all patients and relapse rate as a percentage of initially cured patients.

Trial	Treatment	Number of patients	Cure [%]	Recurrence [%]	Sustained response [%]
[76]	vancomycin 125 mg qid, 5 days	9	78	0	78
	placebo	7	14	-	-
	No clear case definition. No description of allocation of treatment. Only data of patients with toxin-positive stool shown. Unclear length of follow-up and incidence or relapse in placebo group. $p < 0.02$ for comparison of cure rates.				
[77]	vancomycin 500 mg qid, 10 days	32	100	19	81
	metronidazole 250 mg qid, 10 days	32	97	6	91
	Only data of patients with toxin-positive stools or pseudomembranous colitis shown. Per-protocol analysis. Follow-up 21 days. Differences not statistically significant.				
[78]	vancomycin 125 mg qid, 7 days	21	86	33	58
	bacitracin 20000 U qid, 7 days	21	76	42	44
	Double-blind. 25% drop-out during follow-up of bacitracin group. Follow-up 5 weeks. Differences not statistically significant.				
[79]	vancomycin 500 mg qid, 10 days	15	100	20	80
	bacitracin 25000 U qid, 10 days	15	80	42	46
	Double-blind. Patients had leukocytosis, fever or abdominal pain. 29% drop-out in vancomycin group, 12% in bacitracin group. Per-protocol analysis. Unclear definition of failure ('worsening during treatment'). Failing patients crossed over to alternate drug. Interruption of study drug in vancomycin group for a mean of 2.8 days and in bacitracin group for a mean of 1.8 days. Unclear length of follow-up. Differences not statistically significant.				
[80]	vancomycin 125 mg qid, mean 10.6 days	24	100	21	79
	vancomycin 500 mg qid, mean 10.1 days	22	100	18	82
	Variable duration of therapy. 18% dropout rate. Per-protocol analysis. Unclear length of follow-up. Differences not statistically significant.				
[81]	vancomycin 500 mg bid, 10 days	10	100	-	-
	rifaximin 200 mg tid, 10 days	10	90	-	-
	Article in Italian. Patients had diarrhoea, abdominal pain and fever. No description of allocation of treatment. Unclear definition of cure. Differences not statistically significant.				

Tabel 8 Continued.

Trial	Treatment	Number of patients	Cure [%]	Recurrence [%]	Sustained response [%]
[82]	vancomycin 500 mg qid, 10 days	20	100	20	80
	teicoplanin 100 mg bid, 10 days	26	96	8	88
	No description of allocation of treatment. Per-protocol analysis. Unclear length of follow-up ('at least 1 month'). Differences not statistically significant.				
[83]	teicoplanin 100 mg qid, 3 days, followed by 100 mg bid, 4 days	24	96	35	62
	teicoplanin 100 mg bid, 7 days	23	70	50	35
	Double-blind. Outcome of 'improvement, but not cure' (2 loose stools per day or 1 loose stool per day with fever or cramps) was counted as failure. 3 patients with improvement in bid group; 1 in qid group. Follow-up 5 weeks. $p = 0.08$ for comparison of cure rates.				
[84]	vancomycin 500 mg tid, 10 days	31	94	17	78
	metronidazole 500 mg tid, 10 days	28	96	7	89
	teicoplanin 400 mg bid, 10 days	29	93	30	65
	fusidic acid 500 mg tid, 10 days				
	Follow-up 30 days. Only statistically significant difference was relapse rate of fusidic acid versus teicoplanin ($p = 0.042$).				
[85]	metronidazole 400 mg tid, 7 days	55	93	30	65
	fusidic acid 250 mg tid, 7 days	59	83	30	58
	Double-blind. 13% drop-out during treatment; 15% further drop-out during follow-up. Per-protocol analysis. Follow-up 35 days. Differences not statistically significant.				
[86]	metronidazole 250 mg qid, 10 days	34	82	30	57
	nitazoxanide 500 mg bid, 7 days	40	90	26	67
	nitazoxanide 500 mg bid, 10 days	36	89	16	75
	No definition of relapse. Double-blind. 23% drop-out during treatment. Per-protocol analysis. Follow-up 31 days. Differences not statistically significant.				
[87]	metronidazole 500 mg tid, 10 days	20	65	38	40
	metronidazole 500 mg tid + rifampicin 300 mg bid, 10 days	19	63	42	37
	Intention-to-treat analysis. Follow-up 40 days. Differences not statistically significant.				

Tabel 8 Continued.

Trial	Treatment	Number of patients	Cure [%]	Recurrence [%]	Sustained response [%]
[88]	vancomycin 125 mg qid, 10 days	71	97	7	90
	metronidazole 250 mg qid, 10 days	79	84	14	72
	Double-blind. 13% drop-out during treatment. Per-protocol analysis. Follow-up 21 days. p = 0.006 for comparison of cure rates. p = 0.27 for comparison of relapse rates. The original protocol was stratified in a group with mild and a group with severe disease (based on age, fever, albumin level and leukocyte count), which resulted in a larger difference between cure rates in the group with severe disease and a statistically non-significant difference between cure rates in the group with mild disease. Intention-to-treat analysis with dropouts regarded as failures resulted in a statistically significant difference between overall cure rates (initial cure minus relapse; 57 out of 90 versus 64 out of 82; risk ratio 0.91). Other comparisons were not significant anymore in the intention-to-treat analysis.				
[89]	fidaxomicin 50 mg bid, 10 days	14	71	8	65
	fidaxomicin 100 mg bid, 10 days	15	80	0	80
	fidaxomicin 200 mg bid, 10 days	16	94	6	88
	Open-label. Patients with signs of highly severe CDI (> 12 bowel movements per day, vomiting, severe abdominal tenderness, ileus, WBC > 30, toxic megacolon) were excluded. Cure = complete resolution of diarrhoea. Follow-up 6 weeks after end of treatment.				
[90]	vancomycin 125 mg qid, 10 days	27	74	7	69
	nitazoxanide 500 mg bid, 10 days	22	77	5	73
	CDI = stool EIA for toxin A or B positive AND (temperature > 38.3 ºC OR abdominal pain OR leukocytosis). Patients with > 1 episode in preceding 6 months were excluded. 12% dropout rate during treatment. Double-blind, placebo-controlled. Modified intention-to-treat analysis. Industry-sponsored. Cure = complete resolution of symptoms during 3 days after completion of therapy. Per-protocol analysis: 87 vs. 94% cure. Follow-up 31 days after start of treatment. No differences in severity subgroups. Differences not statistically significant.				
[70]	vancomycin 125 mg qid, 10 days	309	86	25	65
	fidaxomicin 200 mg bid, 10 days	287	88	15	75
	Placebo-controlled. Industry-sponsored. Very severe CDI and more than one previous episode excluded. Designed as non-inferiority trial. 4 weeks follow-up for recurrences after completion of study drug. Cure = < 4 times daily passage of unformed stools AND no necessity for additional treatment. Fidaxomicin was not associated with fewer recurrences in CDI due to PCR ribotype 027 as opposed to non-027. Modified intention-to-treat (patients who received at least one dose of the study drug) and per-protocol analyses were similar.				
[91]	vancomycin 125 mg qid, 10 days	257	87	27	64
	fidaxomicin 200 mg bid, 10 days	252	88	13	77
	Methods identical to the trial by Louie [32]. Contrary to that trial, this trial did show fewer recurrences in both PCR ribotype 027 and non-027 patients, although the difference was not significant for the former subgroup.				

Tabel 9 Observational studies of oral antibiotic treatment of initial *Clostridium difficile* infection (CDI). Initial cure rate and sustained response as a percentage of all patients and relapse rate as a percentage of initially cured patients

Trial	Treatment	Number of patients	Cure [%]	Recurrence [%]	Sustained response [%]
Antibiotics:					
[113]	vancomycin	79	96	14	83
[114]	vancomycin	16	100	13	87
[115]	metronidazole	13	100	15	85
[116]	vancomycin	189	97	24	74
[106]	vancomycin 500 mg qid, 10 days	23	100	13	87
	teicoplanin 200 mg bid, 10 days	22	100	0	100
[117]	metronidazole	632	98	6	92
	vancomycin	122	99	10	89
[57]	metronidazole	44	?	50	-
[118]	metronidazole	99	62	?	-
[119]	metronidazole	207	78	28	56
[68]	metronidazole	1123	84	29	60
	vancomycin	112	?	28	-
[120]	fidaxomicin varying dose	45	91	5	86
[121]	nitazoxanide 500 mg bid, 10 days	35	74	27	54
	Patients first failed metronidazole.				
[101]	metronidazole*	34	>90	12	>79
	*Ten patients switched to vancomycin.				
	vancomcyin	18	>90	11	>80
[122]	tigecycline varying duration	4	100	0	100
	Severe CDI. Follow-up at least 3 months.				
[123]	rifaximin 400 mg tid	8	100	10	90
	2 weeks follow-up.				

Oral vancomycin has been shown to be poorly absorbed in most patients, usually producing minimal or subtherapeutic serum concentrations. However, bowel inflammation may enhance absorption of oral vancomycin, particularly in those with renal failure, thereby increasing the risk for systemic side-effects [110]. A recently performed safety analysis of fidaxomicin in comparison with oral vancomycin revealed no differences in serious adverse events between these agents [111]. Fidaxomicin is minimally absorbed. While no specific concerns related to hypersensitivity reactions were identified during the drug development, hypersensitivity reactions associated with fidaxomicin use have been reported to the FDA in the post-marketing phase. The fidaxomicin labeling was revised to include information about the possibility of hypersensitivity reactions [112].

To evaluate the clinical outcomes of the main antimicrobial agents used in the treatment of CDI, we compared dosages, cure rate, recurrence rate, stated time to response and adverse events of treatment with vancomycin, metronidazole and fidaxomicin. Only randomized controlled trials of antibiotic treatment of initial CDI were included. Results are summarized in Table 10.

Recommendations. In case of non-severe CDI (no signs of severe colitis) in non-epidemic situations and with CDI clearly induced by the use of antibiotics, it may be acceptable to stop the inducing antibiotic and observe the clinical response for 48 h, but patients must be followed very closely for any signs of clinical deterioration and placed on therapy immediately if this occurs. Metronidazole is recommended as oral antibiotic treatment of initial CDI in mild/moderate disease. For detailed recommendations on oral antibiotic treatment of initial non-severe CDI refer to Table 11.

Alternative treatment regimens treatment for non-severe disease

Evidence. Tables 12 and 13 report the evidence from randomized trials and observational studies on the non-antibiotic treatment of initial CDI, with comments on methodology. The majority of these alternative treatment strategies are combined with antibiotic treatment. Currently there are no randomized controlled trials on the use of human intravenous gammaglobulins (IVIG). Passive immunizations with IVIG have been reported to be successful in small case series, but the grade of evidence and strength of recommendation of IVIG are too weak to allow recommendations on the use of IVIG in CDI [4,130]. Hypogammaglobulinaemia, e.g. following solid organ transplants, may predispose to CDI. For this subgroup of patients, IVIG may be beneficial, but more studies are needed before this can be recommended definitively [4]. A recent systematic review on the use of probiotics suggests that probiotics are associated with a reduction in antibiotic-associated diarrhoea [131]. A recent meta-analysis on probiotic prophylaxis for CDI, concluded that moderate-quality evidence suggests a beneficial effect of probiotic prophylaxis in CDI without an increase in clinically important adverse events [132]. However, a Cochrane analysis concluded

Table 10 Results of randomized controlled trials of oral antibiotic treatment of initial Clostridium difficile infection (CDI) with vancomycin/teicoplanin, metronidazole and fidaxomicin: comparison of dosages, cure rate, recurrence rate, stated time to response or adverse effects due to treatment

	Trial	Number of patients	Dosages and duration of therapy	Time to initial response (mean)	Cure rate [%]	Recurrence rate [%] and definition	Adverse events [%]
Vancomycin	[76]	9	125 mg qid 5 days	-	78	0 Recurrence not defined, follow-up period not specified	-
	[77]	32	500 mg qid 10 days	3.2 days	100	19 Reappearance of diarrhoea < 21 d after therapy	3 Drug intolerance
	[78]	21	125 mg qid 7 days	-	86	33 Reappearance of diarrhoea < 5 wk after therapy	-
	[79]	15	500 mg qid 10 days	-	100	20 Reappearance of diarrhoea after therapy Follow-up: length not clear	-
	[80]	24	125 mg qid mean 11 days	4 days	100	21	0
		22	500 mg qid mean 10 days	4 days	100	18 Recurrence of disease not further specified Follow-up not defined	0

Table 10 Continued

	Trial	Number of patients	Dosages and duration of therapy	Time to initial response (mean)	Cure rate [%]	Recurrence rate [%] and definition	Adverse events [%]
	[81]	10	500 mg bid 10 days	3.8 days	100	? Not described No follow-up period	0
	[82]	20	500 mg qid 10 days	3.6 days	100	4 Reappearance of diarrhoea and other symptoms ≥ 1 m after therapy. Follow-up not further specified	0
	[84]	31	500 mg tid 10 days	3.1 days	94	17 Reappearance of diarrhoea and other symptoms < 25-30 d after therapy	0
	[88]	71	125 mg qid 10 days	-	97	7 Recurrence of CD toxin positive diarrhoea within 21 d after start of therapy	1 (nausea)
	[90]	27	125 qid 10 days	Median: 96 hr (estimated from Graph)	74	7 Return of symptoms (toxin positive diarrhoea) < 31 d after onset of treatment, or clinical response after empiric re-treatment for CDI	0
	[70]	30	125 mg qid 10 days	Median: 78 hr	86	25 Reappearance of CD toxin positive diarrhoea < 4 wk after treatment and need for retreatment for CDI	Possibly or definitely related: 9 Serious events related to laboratory test results: 1.2
	[91]	257	125 mg qid 10 days	Median: 60 hr (estimated from Graph)	87	27 Return of CD toxin positive diarrhoea < 30 d after treatment and need for retreatment for CDI	Any treatment-emergent adverse event related to study drug: 13.8
Teicoplanin	[82]	26	100 mg bid 10 days	3.4 days	96	2 Reappearance of diarrhoea and other symptoms ≥ 1 m after therapy. Follow-up not further specified	0
	[84]	28	400 mg bid 10 days	2.8 days	96	7 Reappearance of diarrhoea and other symptoms < 25-30 d after therapy	0
	[83]	24	100 mg qid, 3 days, followed by 100 mg bid, 4 days 100 mg bid 7 days		96	35	7-8 % vomiting, nausea, exanthema, arthralgia, pruritus, hallucinations. No abnormal laboratory results
		23			70	50	
Metronidazole	[77]	32	250 mg qid 10 days	3.1 days	97	6 Reappearance of diarrhoea < 21 d after therapy	3
	[84]	31	500 mg tid 10 days	3.2 days	94	17 Reappearance of diarrhoea and other symptoms < 25-30 d after therapy	10 GI discomfort
	[85]	55	400 mg tid 7 days	Within 5 days	93	30 Reappearance diarrhoea during 28-33 d after treatment	14.5 GI, exanthema, taste

Table 10 Continued

	Trial	Number of patients	Dosages and duration of therapy	Time to initial response (mean)	Cure rate [%]	Recurrence rate [%] and definition	Adverse events [%]
	[86]	34	250 mg bid 10 days	Median: 3 days (estimated from Graph)	82	30 Reappearance of symptoms < 31 days after start of treatment and after at least 1 negative CD toxin test before retreatment	related to study drug:0 serious adverse events not related to study drug:18.2 intolerance or allergy:0
	[87]	20	500 mg tid 10 days	6.6 days	65	38 Recurrence of diarrhoea < 30 d after treatment	40 (not specified if related to study drug: rash, nausea vomiting)
	[88]	79	250 mg qid 10 days	Not specified	84	14 Recurrence of CD toxin positive diarrhoea < 21 d after start of therapy	1.3 (nausea)
Fidaxomicin	[89]	14 15 16	50 mg bid 10 days 100 mg bid 10 days 200 mg bid 10 days	Median 6.3 Median 4.8 Median 3.6	71 80 94	8 0 6 Recurrence of CD toxin positive diarrhoea <6 wk after treatment	20% but not related to study drug.
	[70]	287	200 mg bid 10 days	Not reported	88	15 Reappearance of CD toxin positive diarrhoea <4 wk and need for retreatment for CDI	Possibly or definitely related: 9.7 Serious events related to laboratory test results: 4.7
	[91]	252	200 mg bid 10 days	Not reported	88	13 Return of CD toxin positive diarrhoea < 30 d and need for retreatment for CDI	Any treatment-emergent adverse event related to study drug: 11.7

Table 11 Recommendations on oral antibiotic treatment of initial Clostridium difficile infection (CDI): mild/moderate disease

Treatment	SoR	QoE	Reference(s)	Comment(s)
Stop inducing antibiotic(s) and observe the clinical response for 48 hrs	C	II	[116,117]	Rate of spontaneous resolution unknown in mild CDI. Studies performed before increased incidence of hypervirulent strains.
Metronidazole 500 mg tid 10 - 14 days	A	I	[77,84–88]	No statistally significant difference in cure rate between metronidazole and vancomycin or teicoplanin. Statistically significant difference in sustained clinical cure between metronidazole and vancomycin in favour of vancomycin in one study [2,62] (and pooled results of two unpublished randomized controlled trials)
Vancomycin 125 mg qid 10 days or Teicoplanin 100 mg bid 10 days	B	I	[70,76,78,80,82,84,88,90,91]	Teicoplanin significantly better than vancomycin for bacteriologic cure and borderline superior in terms of symptomatic cure [2]
Vancomycin 500 mg qid 10 days or Teicoplanin 400 mg bid 10 days	C	I	[77,79–82,84]	Vancomycin: Equal cure rate 500 mg compared to 125 qid [54] BI Teicoplanin: one dose finding study: 50 mg qid superior to 100 mg bid. [57] No significant differences in cure-rate or recurrence-rate between studies using 400 mg bid and 100 mg bid respectively: [56,58]
Fidaxomicin 200 mg bid 10 days	B	I	[70,89,91]	Industry sponsored studies. Fewer recurrences as compared to vancomycin. [65]

Table 12 Randomized controlled trials of non-antibiotic treatment of initial Clostridium difficile infection (CDI). Initial cure rate and sustained response as a percentage of all patients and relapse rate as a percentage of initially cured patients

Trial	Treatment	Number of patients	Cure [%]	Recurrence [%]	Sustained response [%]
Probiotics:					
[126]	vancomycin or metronidazole + *Saccharomyces boulardii* $2{\cdot}10^{10}$ CFU/ day, 4 weeks	31	-	19	-
	vancomycin or metronidazole + placebo	33	-	24	-
	Double-blind. No control for type, duration or dose of antibiotic. Unclear definition of relapse. Follow-up 8 weeks after start of treatment. p = 0.86 for comparison of relapse rates.				
Toxin-binding resins and polymers:					
[24]	tolevamer 1 g tid, 14 days + placebo	94	60	16	50
	tolevamer 2 g tid, 14 days + placebo	91	79	7	74
	vancomycin 125 mg qid, 10 days + placebo	94	91	19	74
	Non-inferiority trial. Patients with stool frequency > 12 per day or abdominal pain were excluded. Tolevamer could be prolonged when inciting antibiotic could not be stopped. Double-blind. 23% drop-out. Per-protocol analysis. Cure rate of tolevamer 2g non-inferior in comparison with vancomycin (Chow-test p = 0.03). Non-inferiority of tolevamer 1g compared with vancomycin could not be demonstrated. p = 0.05 for comparison of relapse rates of tolevamer 2g with vancomycin. Relapse rates of tolevamer 1g and vancomycin not statistically different. Follow-up 6 – 8 weeks.				
[124]	tolevamer 3g tid, 14 days	266	47	3	46
	vancomycin 125 mg qid, 10 days	134	81	23	62
	metronidazole 375 mg qid, 10 days	143	72	27	53
	Unpublished trial.				
[125]	tolevamer 3g tid, 14 days	268	42	6	40
	vancomycin 125 mg qid, 10 days	125	81	18	66
	metronidazole 375 mg qid, 10 days	135	73	19	59
	Unpublished trial.				
Immunotherapy:					
[71]	single dose of 10 mg/kg CDA1 and CDB1 (iv. administered human monoclonal antibodies against TcdA and TcdB) with standard antimicrobial therapy	101	93	7	87
	placebo with standard antimicrobial therapy	99	87	25	65
	Industry-sponsored and -analyzed. Patients must have diarrhea and receive vancomycin or metronidazole at time of enrollement. Diarrhea = >2 unformed stools on 2 consecutive days or >6 unformed stools on 1 dat. Recurrence = new episode of diarrhea with new positive stool toxin test after resolution of initial diarrhea. Analysis for recurrence only performed in those who were cured, received >7 days of antimicrobial therapy and did not receive IVIG (93 vs. 82). Dropout rate 9 vs. 13%, mainly due to deaths not related to CDI. Vancomycin: 30 vs. 22%. Follow-up 12 weeks. p < 0.001 for comparison of relapse rates. Intention-to-treat analysis. Primary endpoint was changed during the study before unblinding. Orginal endpoint: resolution of illness. Subgroup analysis: similar results, although difference much smaller in inpatients than outpatients. Length of hospitalisation did not differ.				

Table 13 Observational studies of non-antibiotic treatment of initial Clostridium difficile infection (CDI). Initial cure rate as a percentage of all patients and relapse rate as a percentage of initially cured patients

Trial	Treatment	Number of patients	Cure [%]	Recurrence [%]
Toxin-binding resins and polymers:				
[127]	colestipol 10 g qid, 5 days	12	25	-
	Originally set up as a randomized placebo-controlled trial. Placebo group was merged with historical control, however. Only 6 patients had toxin-positive stool..			
Passive immunotherapy with immune whey:				
[128]	metronidazole or vancomycin followed by immune whey protein concentrate, 14 days	16	100	0
	56% of patients had recurrent CDI; mean follow-up 333 days.			
[129]	metronidazole or vancomycin followed by immune whey protein concentrate, 14 days	109	100	10
	109 episodes; 101 patients; 40% of patients had recurrent CDI.			

that there was insufficient evidence to recommend probiotics, in general, as an adjunct to antibiotics in the treatment of *C. difficile* diarrhoea [133]. Although no cases of translocation of microorganisms have been reported in clinical trials with probiotics for antibiotic-associated diarrhoea or CDI, probiotics should be used with caution. Several studies of invasive disease have been reported, resulting from the use of probiotics such as *Saccharomyces boulardii* in debilitated or immunocompromised patients [134,135]. Moreover, probiotics were associated with increased mortality, partly due to non-occlusive mesenteric ischaemia, in a randomized controlled trial in acute pancreatitis [136].

Recommendations. There is insufficient evidence to support administration of probiotics, toxin-binding resins and polymers, or monoclonal antibodies. For detailed recommendations refer to Table 14.

B: Severe *Clostridium difficile* Infection

Oral antibiotic therapy

Evidence. In 6/17 randomized controlled trials, severity of disease was defined. Definitions varied among the studies. Only in 4/6 of these trials were treatment results specified for severity of disease (Table 15).

Recommendations. Based on its pharmacokinetic properties vancomycin is considered superior to metronidazole in severe *C. difficile* disease [22,88]. The use of high doses of vancomycin (500 mg orally four times daily) was included in the Infectious Diseases Society of America/Society for Healthcare

Epidemiology of America treatment guidelines [3] for management of severe complicated CDI as defined by the treating physician. However, there is insufficient evidence to the use of doses >125 mg four times daily in the absence of ileus [80].

Fidaxomicin was not inferior to vancomycin for initial cure of CDI, but there are no data available on the efficacy of this drug in severe life-threatening disease [70,91]. For detailed recommendations on oral antibiotic treatment of severe CDI refer to Table 16.

Surgery for complicated *Clostridium difficile* infection

Evidence. Patients with fulminant CDI who fail to respond and who progress to systemic toxicity, peritonitis, or toxic colonic dilatation and bowel perforation require surgical intervention [4]. Mortality rates of emergency surgery in complicated CDI remain high, ranging from 19% to 71% depending on the clinical condition of the patient at the time of surgery [138]. However, recently as systematic review of the existing literature was performed to assess the effect on mortality of colectomy for the treatment of fulminant CDI. The authors concluded that colectomy is associated with

Table 14 Recommendations on alternative treatment regimens for initial Clostridium difficile infection (CDI)

Type of intervention	Treatment	SoR	QoE	Reference(s)	Comment(s)
Probiotics	Vancomycin or metronidazole + *Saccharomyces boulardii*	D	I	[126,137]	Comparison of relapse rates: in subgroup analysis efficacy in recurrent CDI, but *not* in initial CDI. Evidence-based review: [137].
Toxin binding resins and polymers	Tolevamer 3 g tid	D	I	[24]	Industry sponsored studie. Non-inferiority trial: tolevamer vs vancomycin.
Immunotherapy	Human monoclonal antibodies against TcdA and TcdB with standard antimicrobial therapy (metronidazole and vancomycin)	C	I	[48]	Industry sponsored study. Fewer recurrences. Subgroup analysis: BI/NAP1/027 strain, patients with >1 recurrence and hospitalization.
	Passive immunotherapy with immune whey after standard oral antimicrobial therapy	C	II	[129]	Observational study: 101 CDI patients (40% recurrent CDI). Results suggest reduction in recurrence rate.

Table 15 Randomized controlled trials of oral antibiotic treatment of initial Clostridium difficile infection (CDI) in which severity of disease is defined and outcome of treatment is specified for severity of diseases

Study	Treatment	CDI severity: Moderate/ Mild (M), Severe (S) Number of patients (%)	Initial cure Number of patients (%)	Relapse Number of patients (% of patients with initial cure)	Sustained response rate* Number of patients (% of all patients)
[88]	vancomycin	M 40/71 (56)	39/40 (98)	2/39 (5)	37/40 (93)
	125 mg qid, 10 days	S 31/71 (44)	30/31 (97)	3/30 (10)	27/31 (87)
	metronidazole	M 41/79 (52)	37/41 (90)	3/37 (8)	34/41 (83)
	250 mg qid, 10 days	S 38/79 (48)	29/38 (76)	6/29 (21)	23/38 (61)
		Intention to treat analysis:	Intention to treat analysis:	Intention to treat analysis:	Intention to treat analysis:
	vancomycin	M 44/82 (49)	39/44 (89)	2/39 (5)	37/44 (84)
	125 mg qid, 10 days	S 38/82 (46)	30/38 (79)	3/30 (10)	27/38 (71)
	metronidazole	M 46/90 (51)	37/46 (80)	3/37 (8)	34/46 (74)
	250 mg qid, 10 days	S 44/90 (49)	29/44 (66)	6/29 (21)	23/44 (52)
[90]	vancomycin	M 17/27 (63)	13/17 (76)	1/13 (8)	12/17 (71)
	125 mg qid, 10 days	S 10/27 (37)	7/10 (70)	1/7 (14)	6/10 (60)
	nitazoxanide	M 12/22 (55)	9/12 (75)	0/9 (0)	9/12 (75)
	500 mg bid, 10 days	S 10/22 (45)	8/10 (80)	1/8 (13)	7/10 (70)
[70]	vancomycin	M 186/309 (60)	156/186 (85)	38/156 (24)	118/186 (63)
	125 mg qid, 10 days	S 123/309 (40)	109/123 (89)	29/109 (27)	80/123 (65)
	fidaxomicin	M 175/287 (61)	161/175 (92)	27/161 (17)	134/175 (77)
	200 mg bid, 10 days	S 112/287 (39)	92/112 (82)	12/92 (13)	80/112 (71)
[91]	vancomycin	M 196/257 (76)	180/196 (92)	46/180 (26)	134/196 (68)
	125 mg qid, 10 days	S 61/257 (24)	43/61 (71)	14/43 (33)	29/61 (48)
	fidaxomicin	M 189/252 (75)	173/189 (92)	24/173 (14)	149/189 (79)
	200 mg bid, 10 days	S 63/252 (25)	48/63 (76)	4/48 (8)	44/63 (70)

*Sustained response rate: clinical cure and no recurrences during follow up

Table 16 Recommendations on oral antibiotic treatment of initial Clostridium difficile infection (CDI): severe disease

Treatment	SoR	QoE	Reference(s)	Comment(s)
Vancomycin, 125 mg four times daily for 10 days	A	I	[70, 88, 90, 91]	Cure rate higher as compared with metronidazole in severe CDI [88][a]
Vancomycin 500 mg four times daily for 10 days	B	III (I*)	[80]	Randomized controlled trial on dose effectiveness: no significant differences in measurable responses of high-dose compared to low-dose regimens. However: results not stratified for severity of illness [80][a].
Fidaxomicin 200 mg twice daily for 10 days	B	I	[70,89,91]	Evidence limited to two Phase III studies [70,91]. Fewer recurrences compared with vancomycin 125 mg four times daily in severe disease (except for PCR ribotype 027). No data on the efficacy in severe life-threatening disease and/or toxic megacolon: excluded from both studies.
Metronidazole, 500 mg three times daily for 10 days	D	I	[88]	Cure rate lower as compared with vancomycin in severe CDI [88]. Intention to treat analysis not reported. Extremely severe CDI excluded[a]. Differences in symptomatic cure of metronidazole versus vancomycin not statistically significant in a pooled analysis [2]. ICU admission and hypoalbuminaemia (= disease severity) predictors of metronidazole failure [119].

aTwo studies reported in abstract form confirm the superiority of vancomycin over metronidazole for treatment of (severe) CDI [92,124,125].

a lower mortality than continued medical treatment when this is no longer improving the patient [139]. Several studies suggest that earlier colectomy (time from presentation to surgery) is associated with improved survival [140]. Independent risk factors for mortality in patients who underwent colectomy that have been found among multiple studies include: the development of shock (need for vasopressors), increased serum lactate (≥5 mM), mental status changes, end organ failure, renal failure and the need for preoperative intubation and ventilation [29,35,138,141,142]. The more negative prognostic signs a patient has, the earlier surgical consultation and operative management should be considered. The established operative management of severe, complicated CDI has been subtotal colectomy with end-ileostomy [140]. However, recently an alternative surgical treatment with creation of a diverting loop ileostomy, followed by colonic lavage, has been shown to reduce morbidity and mortality, while preserving the colon. The surgical approach involves the laparoscopic creation of a diverting loop ileostomy. The colon is then lavaged in an ante-grade fashion through the ileostomy with a high volume of polyethylene glycol 3350 or balanced electrolyte solution and the effluent is collected via a rectal drainage tube. A catheter is placed in the efferent limb of the ileostomy to deliver vancomycin flushes in an antegrade fashion in the postoperative period. In addition, patients receive intravenous metronidazole for 10 days [143]. A multicentre randomized controlled trial is currently being conducted to provide level I evidence for possible implementation of this new treatment into standard practice [http://clinicaltrials.gov/show/ NCT01441271].

Recommendations. Total abdominal colectomy should be performed to treat CDI in case of

- Perforation of the colon
- Systemic inflammation and deteriorating clinical condition despite maximal antibiotic therapy; this includes the clinical diagnoses of toxic megacolon, acute abdomen and severe ileus. Colectomy should preferably be performed before colitis becomes very severe. Serum lactate may, inter alia, serve as a marker for severity (operate before lactate exceeds 5.0 mM).

A future alternative to colectomy may be diverting loop ileostomy and colonic lavage, combined with antibiotic treatment (intracolonic antegrade vancomycin and intravenous metronidazole).

C: First Recurrence or (Risk of) recurrent *Clostridium difficile* infection

Oral antibiotic therapy

Evidence. In 3/17 randomized controlled trials of antibiotic treatment of initial CDI, results were specified for CDI before the study (Table 17).

Recommendations. The incidence of a second recurrence after treatment of a first recurrence with oral metronidazole or vancomycin is similar. Fewer secondary recurrences with oral fidaxomicin as compared with vancomycin after treatment of a first recurrence are reported [70,91,144]. However, the evidence on fidaxomicin for this specific subgroup of CDI patients is limited to two phase III studies and based on a retrospective subset analysis of data and a limited number of patients (number of patients in the modified intention-to-treat analysis: fidaxomicin n = 79 and vancomycin n = 80) [144]. There are no prospective randomized controlled trials performed with metronidazole, vancomycin or fidaxomicin in this specific patient group. In addition, fidaxomicin was not associated with fewer recurrences in CDI due to PCR ribotype 027 as opposed to non-027 in one of the randomized controlled trials [70]. Therefore, based on the evidence currently available, the Strength of Recommendation for treating a first recurrence of CDI with oral vancomycin or oral fidaxomicin is considered equal (B-I), unless disease has progressed from non-severe to severe.

For detailed recommendations on oral antibiotic treatment of mild/moderate initial CDI with risk for recurrent CDI or a first recurrence refer to Table 18.

D: Multiple recurrent *Clostridium difficile* infection

Antibiotic and non-antibiotic treatment strategies

Evidence. Tables 19 and 20 report the evidence from randomized trials and observational studies with comments on methodology.

Recommendations. In non-severe second (or later) recurrences of CDI oral vancomycin or fidaxomicin is recommended. Vancomycin and fidaxomicin are equally effective in resolving CDI symptoms, but fidaxomicin has been shown to be associated with a lower likelihood of CDI recurrence after a first recurrence [104,144]. However, there are no prospective randomized controlled trials investigating the efficacy of fidaxomicin in patients with multiple recurrences of CDI. Vancomycin is preferably administered using a tapered and/or pulsed regimen.

Recently the first randomized controlled trial on faecal enteric instillation has been published: faecal transplantation following antibiotic treatment with an oral glycopeptide is reported to be highly effective in treating multiple recurrent CDI [145]. For detailed recommendations on treatment regimens of multiple recurrent CDI refer to Tables 21 and 22.

Table 17 Randomized controlled trials of antibiotic treatment of initial *Clostridium difficile* infection (CDI) in which relapses are defined and outcome of treatment is specified for CDI before study

		CDI before study, No. of patients	Initial cure No. of patients	Relapse No. of patients	Sustained response rate[a]
Study	Treatment	(%)	(%)	(% with initial cure)	No. of patients (%)
[90]	Vancomycin, 125 mg four times daily, 10 days Nitazoxanide, 500 mg twice daily, 10 days	5/27 (19)	4/5 (80)	1/4 (25)	3/5 (60)
		2/22 (9)	2/2 (100)	1/2 (50)	1/2 (50)
[70]	Vancomycin, 125 mg four times daily, 10 days	54/309 (17)	48/54 (89)	15/48 (31)	33/54 (61)
	Fidaxomicin 200 mg twice daily, 10 days	48/287 (17)	42/48 (88)	9/42 (21)	33/42 (78)
[91]	Vancomycin 125 mg four times daily, 10 days	36/257 (14)	32/36 (89)	11/32 (34)	21/36 (58)
	Fidaxomicin 200 mg twice daily, 10 days analysed in: [144]	40/252 (16)	37/40 (93)	7/37 (19)	30/40 (75)

aSustained response rate: clinical cure and no recurrences during follow up.

Table 18 Recommendations on oral antibiotic treatment of mild/moderate initial CDI with risk for recurrent Clostridium difficile infection (CDI) or first recurrence

Treatment	SoR	QoE	Reference(s)	Comment(s)
Metronidazole 500 mg tid 10 – 14 days	B	I	[27,88]	Recurrence rate: metronidazole not inferior to vancomycin or teicoplanin for treatment of mild or severe primary CDI [2,82,88] or after a first recurrence [27]. Vancomycin significantly more effective in bacteriological cure than metronidazole in recurrent CDI [69]
Vancomycin 125 mg qid 10 days	B	I	[70,82,90,91]	No statistally significant difference in recurrence rate between vancomycin and teicoplanin [2,82,84]
Vancomycin 500 mg qid 10 days	C	III	[80]	One randomized controlled trial on dose effectiveness in primary CDI: no significant differences in responses of high-dose compared to low-dose regimens vancomycin. However results not stratified for recurrent CDI [80]
Fidaxomicin 200 mg bid 10 days	B	I	[70,89,91]	Industry sponsored studies. Fewer secondary recurrences as compared to vancomycin after treatment of a first recurrence.

Table 19 Randomized controlled studies of treatment of recurrent Clostridium difficile infection (CDI)

Trial	Treatment	Number of patients	Failure* [%]
Faecal or bacterial instillation:			
[145]	vancomycin 500 mg qid, 14 days	13	69
	vancomycin 500 mg qid 14 days + bowel lavage	13	77
	vancomycin 500 mg qid , 4 days + bowel lavage + nasoduodenal infusion donor feces 3/16 patients with failure after first donor feces infusion received second infusion from a different donor: 2/3 resolved. Treatment with donor feces was superior to either of the vancomycin regimens (both P<0.001). Open label. No definition of diarrhoea. Study terminated by use of Haybittle-Peto rule at unplanned interim analysis. Fecotherapy group was older, had more co-morbidities, had higher creatinine, had more infections with PCR ribotype 027. Other characteristics were comparable.	16	19
Probiotics			
[126]	vancomycin or metronidazole + *Saccharomyces boulardii* $2{\cdot}10^{10}$ CFU/ day, 4 weeks	26	35
	vancomycin or metronidazole + placebo Double-blind. No control for type, duration or dose of antibiotic. Unclear definition of relapse. Follow-up 8 weeks after start of treatment. p = 0.04 for comparison of failure rates.	34	65

Table 19 Continued.

Trial	Treatment	Number of patients	Failure* [%]
[146]	vancomycin 500 mg qid, 10 days, followed by *Saccharomyces boulardii* $2{\cdot}10^{10}$ CFU/ day, 4 weeks	18	17
	vancomycin 500 mg qid, 10 days, followed by placebo	14	50
	vancomycin 125 mg qid, 10 days, followed by *Saccharomyces boulardii* $2{\cdot}10^{10}$ CFU/ day, 4 weeks	45	51
	vancomycin 125 mg qid, 10 days, followed by placebo	38	45
	metronidazole 1g/day, 10 days, followed by *Saccharomyces boulardii* $2{\cdot}10^{10}$ CFU/ day, 4 weeks	27	48
	metronidazole 1g/ day, 10 days, followed by placebo	26	50
	Follow-up 5 months after completion of study drug. p = 0.05 for the comparison of failure rates in patients who received 500 mg of vancomycin qid. Drop-out in this group was 22%. No further statistically significant differences.		
[147]	metronidazole 400 mg tid, 10 days + *Lactobacillus plantarum* 299v $5{\cdot}10^{10}$ CFU/ day, 38 days	12	42
	metronidazole 400 mg tid, 10 days + placebo	9	67
	Double-blind. 28% drop-out. Follow-up 70 days. Difference not statistically significant..		
[148]	vancomycin or metronidazole followed by *Lactobacillus* GG $6{\cdot}10^{11}$ CFU/ day, 21 days	8	38
	vancomycin or metronidazole followed by placebo	7	14
	Patients blinded. No control for type, duration or dose of antibiotic. Follow-up 60 days after completion of antibiotic. Difference not statistically significant.		
Passive immunotherapy with immune whey:			
[149]	colostral immune whey 200 ml tid + placebo, 14 days	18	44
	metronidazole 400 mg tid + placebo, 14 days	20	45
	Double-blind. Multi-centre trial. Follow-up 70 days. Difference not statistically significant.		

* Non-response or relapse

Table 20 Observational studies for treatment of recurrent Clostridium difficile infection (CDI)

Trial	Treatment	Number of patients	Failure* [%]	Mean follow-up
Antibiotics:				
[150]	Vancomycin taper, 21 days, followed by vancomycin pulse, 21 days	22	0	6 m
[151]	vancomycin 125 mg qid + rifampicin 600 mg bid, 7 days	7	0	12 m
[69]	vancomycin 1 – 2 g/day	14	71	59 d
	vancomycin <1 g/day	48	54	59 d
	vancomycin ≥2 g/day	21	43	59 d
	vancomycin taper	29	31	80 d
	vancomycin pulse	7	14	80 d
	metronidazole <1 g/day	29	45	59 d
	metronidazole 1.5 g/day	5	40	59 d
	metronidazole 2 g/day	2	0	59 d
[152]	vancomycin, 14 days, followed by rifaximin varying dose, 14 days	8	13	233 d
[153]	rifaximin 400 mg tid, 14 days, followed by rifaximin 200 mg tid, 14 days	5	0	310 d
	rifaximin 400 mg tid, 36 days	1	100	-
[154]	rifaximin 400 mg tid, 14 days	25	36	56 d
	Severe CDI excluded. Patients unresponsive to metronidazole 500 mg tid, 5 days. Cure = negative stool PCR for TcdB. All patients had resolution of diarrhea, but no definition or description of how this was measured are given.			

Table 20 Observational studies for treatment of recurrent Clostridium difficile infection (CDI)

Trial	Treatment	Number of patients	Failure* [%]	Mean follow-up
Probiotics:				
[155]	metronidazole or bacitracin, 10 days, followed by *Lactobacillus* GG 10^{10} CFU/day, 7–10 days	5	20	-
[156]	*Lactobacillus* GG $6{\cdot}10^{8}$ CFU/day, 14 days	4	0	11 m
Faecal or bacterial instillation:				
[157]	faecal enema faecal enema n=15, enteric tube n=1	16	19	(5d-3y)
[158]	faecal or bacterial enema 2 faecal and 4 bacterial mixture	6	0	6 m
[159]	rectal tube	7	0	2 y
[160]	faecal instillation through colonoscope or gastrostoma	18	17	-
[161]	lower gastrointestinal tract	6	0	(9-50 m)
[162]	nasogastric tube, median 3 courses 2 patients died: not CDI related, 15/16 cure after firts FT, 1 relapse	16	6	90 d
[163]	faecal enema	5	0	-
Louie 2008, abstract derived from [164]	Rectal catheter	45	4	(≤1 y)
[165]	Colonoscopy, enema Complete resolution of symptoms in 8/16 and marked reduction in 7/16.	16	6	6 wk
[166]	vancomycin 500 mg qid, followed by faecal instillation by nasoduodenal tube or colonoscopy	7	29 0 after repeated infusion	150 d
[167]	Nasogastric tube	12	17	90 d
Borody 2008, abstract derived from [164]	Faecal enema CDI in refractory IBD	6	0	8 wk
[168]	nasogastric tube	15	27	median 4 m
[169]	Colonoscopy	37	8	12 m
[170]	Colonoscopy 1/19 non-responders after 1st FT; all cured after 2nd FT	19	5	27 m
[171]	Enema	7	0	9 m
[172]	Colonoscopy	13	15	5 m
[173]	Colonoscopy	12	0	(3 wk-8 yr)
[174]	gastroscopy or colonoscopy	40	27	80 d
[175]	Colonoscopy	26	8	11 m
[176]	Colonoscopy 7/77 treatment failures within 90 days after treatment (early recurrence). 8/77 recurrence > 90 days after treatment (late recurrence).	77	19	17 m
[177]	faecal enema 5/27 patients had two FT: 2/5 failures	27	7	427 d
[178]	faecal instillation through coloscope Patients with (14) and without (29) IBD. 6/43 patients had two FT: 2/6 failures	43	14	2 m
[179]	Colonoscopy Initial failures were all PCR-ribotype 027.	70	11	1 y
Immunotherapy:				
[180]	iv gammaglobulin 400 mg/kg every 3 weeks, 4 – 6 months	5	0	5 m
[181]	iv gammaglobulin 400 mg/kg day 1 and 21	4	0	7.5 m
	iv gammaglobulin, varying dose	5	40	2.8 m
[56]	iv gammaglobulin 300 to 500 mg/kg, 1 to 6 doses	5	40	86 d
[182]	iv gammaglobulin 150 to 400 mg/kg once	14	71	6.6 m
[183]	iv gammaglobulin 200 to 300 mg/kg once	18	33 (died or colectomy)	-
[184]	iv gammaglobulin 75 to 400 mg/kg, 1 to 5 days	21	57 (died)	-

* Non-response or relapse
§ As reported by Bakken [131]
d = days; m = months

Table 21 Recommendations on oral antibiotic treatment of multiple recurrent *Clostridium difficile* infection (CDI) (> 1 relapse)

Treatment	SoR	QoE	Reference(s)	Comment(s)
Metronidazole 500 mg tid 10 – 14 days	D	IIrt	[69,75]	Retrospective case cohort of two placebo/antibiotic trials: [126,146]. Trend for lower recurrence frequency for high-dose vancomycin and low-dose metronidazole [69]. Systematic review: [75].
Vancomycin 500 mg qid 10-14 days	C	IIrt	[69,75]	Retrospective case cohort of two placebo/antibiotic trials: [126,146]. Trend for lower recurrence frequency for high-dose vancomycin and low-dose metronidazole [69]. Systematic review: [75].
Vancomycin 125 mg qid for 10 days, followed by pulse regimen (125–500 mg/day every 2–3 days) for at least 3 weeks.	B	IIt	[69,150]	Retrospective case cohort of two placebo/antibiotic trials [69]: [126,146]. Observational study: [150]. Expert opinion [3].
Vancomycin 125 mg qid for 10 days, followed by taper regimen: gradually decreasing the dose to 125 mg per day.	B	IIt	[69,150]	Retrospective case cohort of two placebo/antibiotic trials [69]: [126,146]. Observational study: [150]. Expert opinion [3].
Fidaxomicin 200 mg bid for 10-14 days	B	IIrt	[75,144]	Evidence limited to two Phase III studies [70,91]. Retrospective subset analysis: fewer recurrences as compared to vancomycin treatment after first recurrence [144]. Systematic review: [75]. Efficacy after multiple recurrences was not investigated [144].

Table 22 Recommendations on non-antibiotic treatment of recurrent *Clostridium difficile* infection (CDI) (> 1 relapse)

Type of intervention	Treatment	SoR	QoE	Reference(s)	Comment(s)
Fecal or bacterial instillation	Vancomycin 500 mg qid , 4 days + bowel lavage + nasoduodenal infusion donor feces	A	I	[145]	Also many observational studies and meta-analyses. [164,186,189–191].
Probiotics	vancomycin or metronidazole + *Saccharomyces boulardii*	D	I	[126]	Comparison of relapse rates: in subgroup analysis efficacy in recurrent CDI, but not in initial CDI. Evidence-based review: [137].
	Vancomycin or metronidazole + *Lactobacillus spp.*	D	I	[147,148]	Evidence-based review: [137].
Passive Immunotherapy with immune whey	Colostral immune whey	D	I	[149]	Study interrupted early.

E: Treatment of *Clostridium difficile* infection when oral administration is not possible

Evidence. Metronidazole remains the only parenteral antibiotic therapy supported by case series [192]. Intravenous metronidazole (500 mg intravenous three times daily) may be added to oral vancomycin, if the patient has ileus or significant abdominal distension [4,44]. However, there are no randomized controlled trials available to guide this recommendation.

It is still unknown how to best treat patients with ileus due to CDI. There are some anecdotal reports on delivery of vancomycin to the gut by means other than orally, mainly through intracolonic delivery. Questions regarding the efficacy, optimal dosing and duration of treatment with intracolonic vancomycin remain unanswered [193,194]. Prospective clinical trials with other antibiotics, like tigecycline, have not yet been performed to support general use [122,195].

Recommendations. When oral treatment is not possible, parenteral metronidazole is recommended, preferably combined with intracolonic or nasogastric administration of vancomycin. Parenteral tigecycline as salvage therapy is only recommended with marginal strength. For detailed recommendations refer to Table 23.

Summary of definitions

Episode of CDI. A clinical picture compatible with CDI and microbiological evidence of free toxins and the presence of *C. difficile* in stool, without reasonable evidence of another cause of diarrhoea.
or
Pseudomembranous colitis diagnosed during endoscopy, after colectomy or on autopsy.

Clinical pictures compatible with CDI.

Diarrhoea: loose stools, i.e. taking the shape of the receptacle or corresponding to Bristol stool chart types 5–7, plus a stool frequency of three stools in 24 or fewer consecutive hours, or more frequently than is normal for the individual.

Ileus: signs of severely disturbed bowel function such as vomiting and absence of stool with radiological signs of bowel distension.

Toxic megacolon: radiological signs of distension of the colon (>6 cm in transverse width of colon) and signs of a severe systemic inflammatory response.

Severe CDI. Severe or life-threatening CDI is defined as an episode of CDI with (one or more specific signs and symptoms of) severe colitis or a complicated course of

Table 23 Recommendations on non-oral antibiotic treatment of initial Clostridium difficile infection (CDI): mild and severe disease

Patient subgroup	Treatment	SoR	QoE	Reference(s)	Comment(s)
Non-severe disease	Metronidazole iv 500 mg tid iv 10-14 days	A	IIu	[192]	Retrospective uncontrolled study [192]
Severe disease and/ or complicated or refractory CDI	Metronidazole 500 mg tid iv 10-14 days + vancomycin retention enema 500 mg in 100 ml normal saline qid intracolonic	A	IIru	[192-194]	Retrospective uncontrolled study. [192] Systematic review [193-194] Expert opinion [3]
		B	III		
	Metronidazole 500 mg tid iv 10-14 days + vancomycin 500 mg qid oral/ nasogastric tube	A	IIru	[192-194]	Retrospective uncontrolled study. [192] Systematic review [193-194] Expert opinion [3]
		B	III		
	Tigecycline iv	C	III	[122]	Observational study/case report [122]

disease, with significant systemic toxin effects and shock, resulting in need for ICU admission, colectomy or death.

One or more of the following unfavourable prognostic factors can be present without evidence of another cause:

- Marked leucocytosis (leucocyte count >15 9 10 /L)
- Decreased blood albumin (<30 g/L)
- Rise in serum creatinine level (≥133 IM or ≥1.5 times the premorbid level)

Recurrent CDI. Recurrence is present when CDI re-occurs
<8 weeks after the onset of a previous episode, provided the symptoms from the previous episode resolved after completion of initial treatment.

Treatment response. Treatment response is present when after therapy either stool frequency decreases or stool consistency improves and parameters of disease severity (clinical, laboratory, radiological) improve and no new signs of severe disease develop.

Treatment response should be observed daily and evaluated after at least 3 days, assuming that the patient is not worsening on treatment. Treatment with metronidazole, in particular, may result in a clinical response only after 3–5 days. After clinical response, it may take weeks for stool consistency and frequency to become entirely normal.

Summary of treatment recommendations

Strength of Evidence (SoE: I to III) and Strength of Recommendation (SoR: A to D) are shown in brackets. For grading definitions we refer to Tables 1 and 2.

A: Initial *Clostridium difficile* infection: non-severe disease

Non-antibiotic treatment

In non-epidemic situations and with (non-severe) CDI clearly induced by the use of antibiotics, it may be acceptable to stop the inducing antibiotic and observe the clinical response for 48 h, but patients must be followed very closely for any signs of clinical deterioration and placed on therapy immediately if this occurs. (C-II).

Oral antibiotic treatment

Metronidazole orally 500 mg three times daily for 10 days (A-I)
Vancomycin orally 125 mg four times daily for 10 days (B-I) Fidaxomicin orally 200 mg twice daily for 10 days (B-I)

B: Severe *Clostridium difficile* infection

Oral antibiotic treatment

Vancomycin orally 125 mg four times daily for 10 days (A-I)
Fidaxomicin orally 200 mg twice daily for 10 days (B-I)

Notes:

- It can be considered to increase the vancomycin dosage to 500 mg four times daily for 10 days (B-III)
- There is no evidence that supports the use of fidaxomicin in life-threatening CDI (D-III)

The use of oral metronidazole in severe CDI or life-threatening disease is strongly discouraged (D-I).

Surgical treatment

Total abdominal colectomy with ileostomy should be per- formed in case of:

- Perforation of the colon
- Systemic inflammation and deteriorating clinical condition not responding to antibiotic therapy; including toxic mega- colon, an acute abdomen and severe ileus.

Surgical treatment should preferably be performed before colitis becomes very severe. Serum lactate may, inter alia, serve as a marker for severity (operate before lactate exceeds 5.0 mM).

A future alternative to colectomy may be diverting loop ileostomy and colonic lavage, combined with antibiotic treat- ment (intracolonic antegrade vancomycin and intravenous metronidazole).

C: First recurrence or (risk of) recurrent *Clostridium difficile* infection

Oral antibiotic treatment

Fidaxomicin orally 200 mg twice daily for 10 days (B-I) Vancomycin orally 125 mg four times daily for 10 days (B-I) Metronidazole orally 500 mg three times daily for 10 days (C-I)

Note: Fidaxomicin was not associated with fewer recurrences in CDI due to PCR ribotype 027 as opposed to non-027 ribotypes.

D: Multiple recurrent *Clostridium difficile* infection

Oral antibiotic treatment

Fidaxomicin orally 200 mg twice daily for 10 days (B-II) Vancomycin orally 125 mg four times daily for 10 days followed by pulse strategy (B-II)
or
Vancomycin orally 125 mg four times daily for 10 days followed by taper strategy (B-II)

Non-antibiotic treatment in combination with oral antibiotic treatment

For multiple recurrent CDI unresponsive to repeated antibiotic treatment, faecal transplantation in combination with oral antibiotic treatment is strongly recommended (A-I).

E: Treatment of *Clostridium difficile* infection when oral administration is not possible

Antibiotic treatment

Non-severe CDI: intravenous metronidazole 500 mg three times daily for 10 days (A-II).

Severe CDI: intravenous metronidazole 500 mg three times daily for 10 days (A-II) combined with vancomycin retention enema 500 mg in 100 mL normal saline four times daily intracolonic, or combined with vancomycin 500 mg four times daily by oral/nasogastric tube for 10 days (B-III).

A schematic overview of currently available therapeutic regimens for CDI, including the quality of evidence (QoE: I to III) and strength of recommendations (SoR: A to D) are shown in Fig. 1.

Authorship

Four draft versions of this guideline document were written by three authors (SD, MB, EK) and critiqued by the Expert Panel. A consensus was reached, resulting in the final version.

Transparency Declaration

Authors: The authors declare that they have no conflicts of interest.
Expert Panel: All members of the expert group completed a Conflict of Interest Disclosure Form (COI).

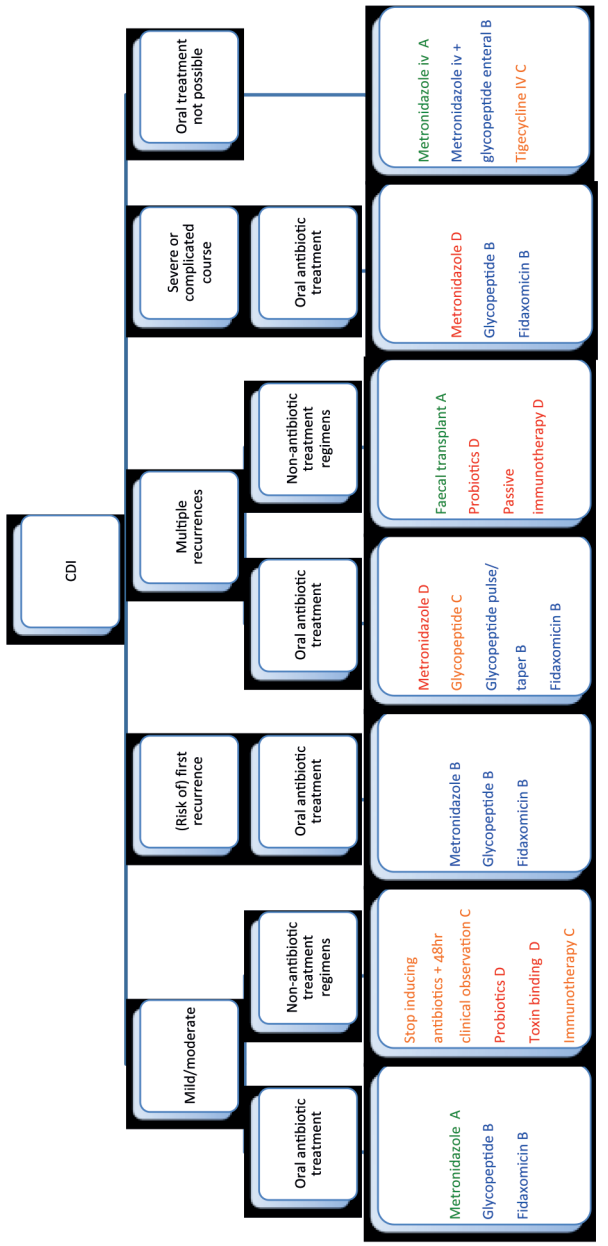

Figure 1 Schematic overview of therapeutic regimens for *Clostridium difficile* infection (CDI). [1]Severe CDI or complicated course: surgical therapy not included in this overview; [2]It can be considered to increase the oral dosage of vancomycin to 500 mg four times daily for 10 days (B-III); [3]There is no evidence that supports the use of fidaxomicin in life-threatening CDI (D-III); Strength of Recommendation (SoR) A = green (Strongly supports a recommendation for use); SoR B = blue (Moderately supports a recommendation for use); SoR C = grey (Marginally supports a recommendation for use); SoR D=red (Recommendation against use).

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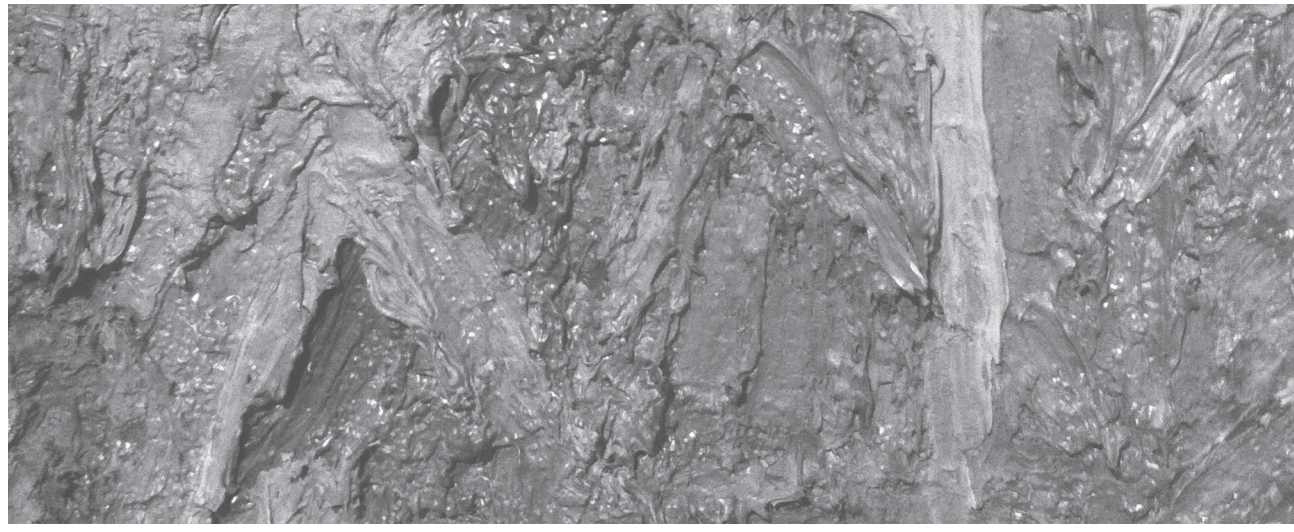
Summary and general discussion

Nederlandse samenvatting

List of publications

Curriculum Vitae



Summary and general discussion

Clostridium difficile, originally named after the difficulty in isolating and characterizing it [1], has proved to be a difficult pathogen indeed, because it has posed us for a number of problems, even more so during the past decade. The importance of various possible sources and transmission routes and the role of asymptomatic carriers are unclear as yet, as are the driving forces behind the emergence and spread of new strains. Some of these strains seem capable of spreading more efficiently, which may in part overlap with a higher virulence. Higher virulence may offer an evolutionary advantage to the bacterium if it results in more cases of refractory disease or recurrences, with ongoing shedding of large amounts of the bacterium and inherent infectiousness. Furthermore, it is currently difficult to identify those patients with a high risk of recurrence and modify management strategies accordingly. The studies described in the preceding chapters aimed to contribute to solving these problems.

In the following paragraphs, the chapters will summarized in numerical order, with a discussion after the summary of each chapter. Chapters 1,2, 3 and 4 concern the epidemiology of *C. difficile* strains. Chapters 5 and 6 concern the prediction of an unfavorable outcome of CDI, particularly recurrence. Chapters 7, 8 and 9 concern the treatment of CDI.

Although *C. difficile* is a ubiquitous microorganism, which may be found in the environment, animals and food, its prevalence is thought to be so much higher in healthcare facilities that for a long time, acquisition of CDI was thought to occur almost exclusively during or shortly after admission to such facilities. The high incidence of CDI in healthcare facilities as compared to the community presumably results from the high density of individuals prone to CDI, typically elderly patients with comorbidity, who may serve as a reservoir in which *C. difficile* can multiply. However, it is increasingly realized that CDI may be acquired outside of healthcare facilities. **Chapters 1 and 2** concern community-onset CDI. In chapter 2, we describe a study that investigated how often one of the large clostridial toxins was found in feces collected from diarrheic patients and submitted to regional diagnostic microbiological laboratories by general practitioners. These feces were submitted for various microbiological diagnostics, most often parasitological testing according to the Dutch general practitioners' guidelines. We demonstrated toxin in the feces of 1.5% of patients. The study was not designed for optimal recovery of the bacterium itself from the feces, but in a large majority of cases, culture was positive as well. Many different strains were encountered, but not – strikingly – the epidemic PCR ribotype 027, in spite of the fact that hospital outbreaks with this strain had occurred in these regions

[2]. Some of the other PCR ribotypes may be considered highly endemic in hospitals or emerging, notably PCR riboptype 078 which was found in four patients, but other strains were rare and not linked to hospitals. The latter finding was supported by the finding that a large number of cases could not be linked to healthcare facilities. Enzyme immunoassays were used to demonstrate the toxin, which had the disadvantage of suboptimal sensitivity, but the advantage of a higher chance that these findings represented CDI and not asymptomatic carriage. This was especially important, since other enteropathogens were not systematically ruled out. Another weakness of this study was selection bias introduced because only feces submitted by general practitioners for testing were investigated. The general practitioners' guideline recommends testing when diarrhea is severe or long-lasting. This may mean that mild community-onset CDI cases were missed. These cases are probably mostly self-limited if they are incited by antibiotics that are subsequently stopped [3].

Several studies have shown a varying proportion of CDI cases to be community-acquired, but the definitions of community acquisition differ. Some studies [4-8] did not systematically examine previous admissions to healthcare facilities. Other studies include a certain period that the patient has not been admitted to a healthcare facility in the definition. The Ad Hoc *C. difficile* Surveillance Working Group [9, 10] advocated a division of CDI into healthcare-associated and community-associated CDI. The latter is defined as CDI with onset in the community (or in a healthcare facility within 48 hours after admission) in a patient who has not been discharged from a healthcare facility in the previous 12 weeks. If the patient was discharged between four and 12 weeks ago, such a case should be classified as being of indeterminate association. A further difficulty concerning studies on community-acquired CDI is patient selection. The studied patient population may strongly influence the proportion of community-acquired CDI and the extrapolated incidence in the community. Estimation of incidences may also be hampered by uncertainty of the size of the population from which stool samples were collected, e.g. a hospital's catchment area. Lastly, *C. difficile* cannot always be assumed to be the cause of the diarrhea, since other enteropathogens are usually not ruled out systematically and asymptomatic carriage is a possibility. In 1995, Karlström et al. [11] found 28% of 1888 CDI cases, defined as unique patients with toxin-positive stool samples, from 13 of Sweden's 31 microbiological laboratories, to be community-acquired, defined as community-onset without hospitalization in the preceding four weeks. In the same year, a study in an Irish tertiary hospital [12] showed 11% of 73 subsequent new CDI cases (diarrhea with positive stool cytotoxicity test) to be community-acquired, which was defined as occurring on or within 72 hours of admission without hospitalization in the previous 60 days. In 1999, a Swedish study [13] found 22% of 267 cases of first episodes of CDI to be community-acquired, i.e. without hospitalization in the previous 60 days. A year later a similar proportion of community-acquired CDI was found in another

Swedish study [14]. A hospital-based study in The Netherlands [15], conducted in 2005, found 20% of 81 CDI cases to be community-onset without hospitalization in the preceding four weeks. After the implementation of a state-wide surveillance system in Connecticut in 2006, 60% of 400 evaluable community-onset CDI cases reported that year by acute-care hospitals met the definition of community-acquisition, i.e. no admissions to healthcare facilities in the previous three months [16]. Because of the above-mentioned methodological problems, it is difficult to calculate incidence rates from these figures. A study based on the United Kingdom General Practice Research Database [17] evaluated the incidence of community-acquired CDI (not hospitalized the previous year), using the number of inhabitants registered with general practitioners providing data for this database as a denominator. From 1994 to 2004, the incidence rose from 0 to 18 per 100,000 persons per year. However, the fundamental problem of selection bias remains. When do GPs decide to perform diagnostics for a case of diarrhea and when do they specifically test for CDI? Two studies tried to address the latter problem by testing stool samples submitted by general practitioners irrespective of the requested diagnostic test. The first study [18] showed 9.2% of 703 stool samples from individual patients to be toxin EIA-positive; 53% of these patients had not been hospitalized in the previous four weeks. In the second study [19], stool samples submitted by GPs to two microbiological labs were randomly selected for testing with cytotoxicity assay. Of 2000 samples, 2.1% were cytotoxin-positive and 45% of the corresponding patients had been hospitalized in the previous six months. The findings of this last study were remarkably similar to those of our study.

Few other studies into community-associated CDI used typing strategies. Contrary to our findings, the Swedish studies [13, 14] found similar distributions of PCR ribotypes among nosocomial and community-acquired cases. Farm animals are obvious candidates for a reservoir, since *C. difficile* may be a commensal and pathogen in animals and farm animals are often exposed to antibiotics. Spores might be spread either by direct contact with animals and their stools or by consumption of contaminated meat. Several studies have demonstrated the presence of *C. difficile* in animal faeces and meat. Evidence for farm animals as a reservoir may come from epidemiological associations and typing studies. Wilcox et al. [19] found the incidence of community-onset CDI to be slightly higher in the urban area that was investigated than in the semi-rural area, whereas in our study, the proportion of positive stool samples was highest in the more rural part. In both an earlier Australian study [6] and our study, there were no patients who reported contact with farm animals. However, in the more rural part of the Netherlands, four out of 10 infections in which PCR ribotyping could be performed were caused by PCR ribotype 078, whereas this ribotype was found in neither of the other regions. This ribotype is frequently found in farm animals and has been associated with community-associated CDI in another

Dutch study [20]. Multilocus variable number tandem repeat analysis of PCR-ribotype 078 isolates in this study revealed four clonal complexes to which both porcine and human strains belonged.

Infants, who have been shown to be frequent asymptomatic carriers [21-23], have also been suggested as a community reservoir for *C. difficile*. Interestingly, Wilcox et al [19] showed contact with infants up to two years of age to be significantly associated with community-onset CDI, when cases were compared to age-matched controls with community-onset diarrhea due to other causes (crude OR 8.2).

Although many patients with community-acquired CDI fulfill the classical risk profile, some appear to be younger with less comorbidity and, strikingly, without recent antibiotic use. Dial et al. [24] found that, of 836 patients of 65 years or older without admission to a healthcare facility in the previous 90 days who were hospitalized with CDI as the primary diagnosis, 46% had not used antibiotics in the previous 90 days. Nevertheless, antibiotic use was still a major risk factor when these patients were compared to patients admitted with other diagnoses (RR 10.6, 95%CI 8.9 – 12.8). Other studies, including ours, found similar proportions of patients without prior antibiotic use. This is a striking difference with studies into nosocomial CDI, which usually show that a large majority of patients have used antibiotics.

From the existing literature and our findings (and partly also those described in chapter 3), we conclude that numerous different strains of *C. difficile* circulate in the community and intermittingly colonize humans, which may lead to disease under the right circumstances. These circumstances are discussed later in this discussion. New strains are probably introduced into healthcare facilities by asymptomatic carriers admitted from the community, as evidenced by a typing study that could not link several nosocomial CDI cases to other cases in the same hospital [25]. It seems likely that humans and animals both act as reservoirs. In order to understand more about the spread of *C. difficile* strains we need large cohorts both inside and outside of the hospital with regular surveillance for *C. difficile* carriage and typing of cultured strains.

In **Chapter 3**, we report a study into asymptomatic carriage by patients with cystic fibrosis (CF). CF, caused by loss-of-function mutations in the gene that encodes the chloride channel, CFTR, leads to a higher viscosity of airway secretions. This in turn leads to stasis, microbial colonization of the airways with chronic inflammation, and bronchiectasis. Depending on the severity of CF, these patients regularly experience respiratory tract infections and therefore are frequently treated with antibiotics and often admitted to hospital. This should make them prone to CDI, but they are thought to seldom develop CDI. Asymptomatic carriage of *C. difficile* has frequently been observed in CF patients who were taking antibiotics [26-28]. No studies on CDI in CF patients have been published since these studies from the early 1980s. Therefore, it

was unclear whether this observation is still valid in our era of emerging epidemic *C. difficile* strains. Furthermore, no typing studies have ever been performed and no risk factors for *C. difficile* carriage in CF carriage have been investigated. Interestingly, a number of cases of severe colitis due to *C. difficile* without diarrhea have been described in CF patients in recent years [29-31]. In our sample of CF patients from one of the CF centers in The Netherlands, we found a high rate of carriage of *C. difficile*, but strikingly, predominantly non-toxigenic strains. Carriage was associated with younger age and more-severe CF. PCR ribotypes were multiple and mostly non-epidemic.

The studies from the 1980s found similarly high rates of *C. difficile* carriage, but only one study found a similarly low proportion of non-toxigenic strains (27%). In two of these studies, carriage was strongly associated with antibiotics. In our study, antibiotics were associated with carriage, but the association was not statistically significant. The majority of CF patients were taking maintenance antibiotics and even more had recently received antibiotic therapy, which probably limited statistical power to detect an association.

Carriage of non-toxigenic *C. difficile* strains probably protects against colonization with toxigenic strains and CDI [32, 33]. These strains circulate in the community [34], but carriage was much rarer among the control group of non-CF patients admitted to an internal medicine ward. A study aiming to colonize healthy volunteers with non-toxigenic *C. difficile* found that it was only possible to establish enduring colonization after administration of antibiotics and repeated doses of non-toxigenic *C. difficile* [35]. This may mean that non-toxigenic strains are able to colonize CF patients more efficiently than they do non-CF patients and thus protect CF patients against CDI. This might be related to a different composition of colonic mucus or microbiome [36]. It may also mean that toxigenic strains have no selective advantage over non-toxigenic strains with respect to colonizing the intestines of CF patients, because the toxins have less effect in CF patients. This latter theory is supported by two of the earlier studies that did find a high proportion of asymptomatic carriage of toxigenic strains in CF patients. If CF patients are protected against large clostridial toxins, could this, too, be related to the composition of their mucus? Or could CFTR be involved in cell entry or cell intoxication by the toxins? A more speculative hypothesis is that due to a presumed higher pH in endosomes of CF patients than that of non-CF patients [37], the conformational change TcdB needs to make in order to cleave itself is inhibited. Elevating the pH of cell organelles protects cells against the effect of large clostridial toxins *in vitro* [38, 39], a finding that we have replicated with 3T3 cells and chloroquine (unpublished data). The fact that CF patients are protected against secretory diarrhea (e.g., caused by *Vibrio cholerae* or enterotoxigenic *E. coli*) by their defective CFTR [40] seems insufficient to explain their low incidence of CDI, as secretion plays only a minor role in the pathophysiology of CDI,

compared to inflammation. Studies to elucidate the mechanism by which CF might protect against CDI could focus on characterizing the microbiome of CF patients, investigating the interaction between colonic mucus from CF patients and *C. dificile* spores, vegetative stages and toxins, studying the effect of clostridial toxins on CFTR knockout cell lines or animals, and studying the prophylactic and therapeutic effect of CFTR inhibitors in CDI in animal models and, if effective and safe, in human CDI.

After the emergence of epidemic PCR ribotype 027 in North America, the UK and The Netherlands, this strain was expected to gain a strong foothold in the rest of Europe. To investigate the distribution of *C. difficile* strains across Europe and morbidity and mortality associated with them, we conducted a study in a selection of European hospitals, described in **Chapter 4**. We found the incidence of CDI to vary widely across European hospitals. Evidently, the small sample size and non-random selection of hospitals do not warrant generalization of the findings to the level of whole countries. However, some findings were striking and led to a number of cautious conclusions. First, PCR ribotype 027 had not become highly prevalent in the hospitals that were studied, and PCR ribotype 078, conspicuously absent in an earlier European survey [41], had become the third most prevalent strain, although the earlier study's different methodology and smaller sample size should be taken into account.

Second, a strikingly high incidence was found in many hospitals in countries bordering the Baltic Sea and North Sea. In Scandinavian countries, consumption of antibiotics per capita is not high, even in the winter months, when respiratory tract infections may lead to more antibiotic use [42]. A lower threshold for testing for CDI might be suggested as an explanation for a higher incidence of CDI, and certainly differences in testing rates were found to correlate with CDI incidence. However, if this difference in testing should account for all of the differences between the various hospitals, this would mean that many cases of hospital-acquired diarrhea were not tested for CDI, contrary to study protocol. Moreover, it would mean that many CDI cases were not noticed, suggesting that these cases were all self-limiting, which is unlikely in hospital-associated CDI. Therefore, the differences in testing rates could also have arisen because there simply were fewer cases of diarrhea due to CDI. If the differences in CDI incidences are true, then they might be explained by differences in patient characteristics between countries, such as severity of comorbidity. The threshold to admit patients to hospital may be higher in countries with well-functioning state-sponsored home care. More speculatively, there may be an association between high CDI incidence and cold or temperate climates. This is especially interesting because the first outbreaks due to PCR ribotype 027 were described in Canada and the northern United States [43]. Also, a seasonal variation of CDI with peaks in late winter - early spring has been described [44-46]. Seasonal variation was not dependent on antibiotic consumption in one study [44]. One may further speculate that circulation of *C. difficile* is somehow increased by low temperatures. The spores can survive high temperatures and prolonged desiccation [47, 48], but it is still possible that they survive longer and in greater numbers in a cold environment. Alternatively, prolonged survival of vegetative bacteria plays a role or more efficient transmission, e.g. because of crowding of humans or animals.

Third, PCR ribotypes 015 and 018 were associated with complications of CDI, mainly mortality for which CDI was judged to be at least partially responsible. It must be stressed that attributability of mortality to CDI is often somewhat subjective - unless patients die of a perforated colon or a toxic megacolon - and mortality due to comorbidities is high in CDI patients. Attributability was therefore left to the judgment of the treating physician, so the associations must be regarded with some caution. PCR ribotype 056 was also associated with a complicated course, but the odds ratio had a wide confidence interval and was only statistically significant after correction for potential confounders. Although this was not indicated by the local hospitals, it is conceivable that some of the cases due to these PCR ribotypes were part of an outbreak, in which mortality due to a specific strain may be higher than in an endemic setting. Especially PCR ribotype 018 was conspicuously present in the Italian hospitals, suggesting rapid spread due to an outbreak. The predominance of PCR ribotype 018 in Italian hospitals was later confirmed by others [49]. Not surprisingly, recurrences were mainly associated with previous recurrences.

In conclusion, predominating *C. difficile* strains in hospitals change over time. What makes a strain outcompete other strains is not entirely clear as yet. The explanation may lie in more efficient transmission due to increased sporulation frequency and spore resistance, differences in germination conditions, lower infectious dose, larger volumes of diarrhea, longer shedding, or differences in antibiotic or disinfectant resistance. Strains may acquire virulence factors and other properties leading to their emergence, as is illustrated by differences between historical and epidemic PCR ribotype 027 [50]. In addition, some strains may be better adapted to patients with certain deficiencies. Coordinated international surveillance may shed more light on the dynamics of the circulation of *C. difficile* strains.

Chapters 5 and 6 describe studies into predictors of the course of CDI, especially a recurrent course. Gaining more insight in the epidemiology of complications and recurrences is important because of two reasons. First, we need predictors of the clinical course of CDI, especially because the prognosis may influence management, such as choice of treatment modality or of alternatives within a specific treatment modality, such as fidaxomicin and vancomycin. These predictors may include epidemiological, clinical, biochemical, radiological and microbiological parameters. Second, such association studies may help to elucidate the pathogenesis of CDI.

The study described in **Chapter 5** investigates the association between one clinical marker (temperature) and two biomarkers (blood leukocyte count and serum creatinine level) with failure of initial therapy for CDI, persistence of diarrhea and recurrence after initially successful therapy for CDI. The data were derived from two large clinical trials that compared fidaxomicin to vancomycin for the treatment of CDI [51, 52]. Failure of initial therapy was partially based on the treating physician's judgment of whether additional therapy was necessary, and therefore less objective than persistence of diarrhea, which was based on the number of unformed bowel movements per day. Failure of therapy was associated with fever, leukocytosis and renal failure, whereas only renal failure was associated with a lower probability of resolution of diarrhea and longer duration of diarrhea. An explanation may be that the treating physician felt a need to start additional therapy for CDI more often when there was fever or leukocytosis. Also, recurrence was only associated with renal failure and not with fever or leukocytosis. The prognostic value of renal failure as a predictor of unfavorable outcome of CDI has been shown previously [53, 54]. Acute renal failure is a common complication of CDI, which is interesting because the volume of diarrhea is not typically large nor is the systemic inflammatory response typically so strong that septic shock with organ hypoperfusion develops. Could there be a direct effect of CDI on renal function? Toxemia has been demonstrated in two children with fatal CDI [55] and in animal models [56]. However, toxemia does not appear to be a common occurrence in human disease.

In this study, we have also shown that leukocyte counts and serum creatinine levels fluctuate around the time of diagnosis of CDI. Follow-up of these parameters after diagnosis of CDI may therefore be important if values are around the upper limit of normal.

In the study described in **Chapter 6**, serum levels of antibodies directed against the large clostridial toxins and other antigens, measured directly after 10 days of antibiotic therapy and three weeks later, were investigated in relation with recurrences during a 60 day follow-up. Also, the relation between recurrences and the neutralizing effect of serum on TcdB was studied. Low serum levels of antibodies against TcdA and TcdB were associated with recurrence, whereas antibodies against other antigens were not, nor was serum neutralizing effect a strong predictor. The main drawback of the study was the fact that sera were taken from patients receiving passive immunotherapy for CDI, and the small number of participants, both of which may have limited the power to detect an association between antibody levels and recurrence. Strikingly, antibody levels often decreased during the three weeks between the two blood samples, which is strange for an immune response directly after an infection and raises the question whether fecal protein loss may have played a role.

Since there was significant overlap in antibody levels between patients who went on to suffer a recurrence and those with a single episode, the conclusion may be drawn that recurrences are not entirely explained by an inadequate humoral immune response. Serious comorbidity was associated with recurrence, but not a specific comorbidity. A possible explanation is that serious comorbidity is associated with changes in the intestinal microbiome, predisposing to recurrences of CDI. Studies that investigate age, comorbidity, antibiotic consumption (measured carefully in defined daily doses, preferably over an extended period), immune response (using the best correlate of mucosal immune response) and microbiome have never been performed. These studies might clarify which of these parameters is associated most strongly with recurrence. The result may have implications for etiological theories of CDI, but also directly for management. The intestinal microbiome may very well be the strongest predictor of recurrence. If so, finding a set of characteristics of the microbiome that can be measured relatively simply in clinical practice would be a worthwhile objective of future studies.

Since antibodies may play a role in the pathogenesis of CDI, the administration of antibodies against *C. difficile* may be an effective strategy to influence the course of the disease. Preferably, these antibodies should be administered enterally, since parenterally administered antibodies may not reach the intestinal lumen, where *C. difficile* resides and produces its toxins. **Chapter 7** reports on a cohort study in which participants who had just recovered from CDI after antibiotic treatment were given an experimental product, derived from milk from cows immunized with toxoid and killed whole-cell *C. difficile,* in order to prevent recurrences. Some of the participants were infected with PCR ribotype 027. Compared to historical controls, participants had a lower recurrence rate, but this is of course no definite proof that the product works. Nonetheless, it seems plausible that passive immunotherapy in the intestinal lumen may be effective by neutralizing toxins and hindering mucosal attachment and cell-cell interactions by the pathogen. Intravenously administered monoclonal antibodies directed against large clostridial toxins were shown to prevent recurrences in one randomized trial [57]. The next step in studying the effectiveness of the milk product should be a randomized placebo-controlled trial. An additional finding of our study was that persistence or reappearance of clostridial toxin in feces after initially successful treatment is associated with relapse. Fecal toxin might be a useful parameter to guide dosage of the experimental product.

For a full overview of all currently available treatment options for CDI, the reader is referred to **Chapters 8 and 9**, which are the first CDI treatment guidance document issued by the European Society of Clinical Microbiology and Infectious Diseases, and a recent update. Currently, the biggest challenge is to find a treatment modality that is as effective in preventing recurrences as fecal transplant, but less cumbersome. Possibly, a mix of bacterial strains may be found as the core component of fecal transplant. This should have the same effect as fecal transplant, but without the

potential for transmission of pathogens, and could be less expensive because there would be no need for screening donors.

The studies described in this thesis aimed to contribute to finding the answers to questions regarding the epidemiology of *C. difficile* strains and the prediction and treatment of CDI with a high risk of recurrence and complications. We feel that we have made a modest contribution by investigating the distribution of *C. difficile* strains in Dutch general practices, among CF patients and in European hospitals. Furthermore, we hope to have shed more light on the etiology and prediction of recurrences and how to minimize the risk of recurrence.

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Nederlandse samenvatting

Inleiding

Ontstaan van ziekte en ziekteverschijnselen

Clostridium difficile is een bacterie die voorkomt in de bodem en in water, maar ook in het maagdarmkanaal van allerlei zoogdieren. De bacterie heeft als kenmerkende eigenschap dat ze in ongunstige omstandigheden over kan gaan in een sporevorm. Deze sporen kunnen jaren overleven onder ongunstige omstandigheden en weer ontkiemen als de omstandigheden gunstiger zijn. Ook de darm van de mens kan gekoloniseerd raken met deze bacterie. De sporen worden ingeslikt en ontkiemen onder invloed van gal in de darm. Als het aantal en de verscheidenheid van de bacteriën in de darm zijn afgenomen, wat vooral onder invloed van antibiotica gebeurt, krijgt *C. difficile* de kans zich te vestigen op het darmslijmvlies. *C. difficile* veroorzaakt ziekte door de toxines TcdA en TcdB te maken, die het darmslijmvlies aantasten. Er ontstaat een ontstekingsreactie waarbij pusvormende witte bloedcellen migreren naar het aangedane gebied in de darm. In het bloed is dit zichtbaar als een verhoging van het aantal witte bloedcellen (leukocytose). Aan het darmslijmvlies is dit met het blote oog te zien als plakken pus op het oppervlak, de zogenaamde pseudo-membranen. De patiënt krijgt diarree en in ernstige gevallen een zeer zieke darm, die doorlaatbaar is voor afbraakproducten van bacteriën met koorts tot gevolg. In uiterste gevallen moet de zieke darm operatief worden verwijderd. De ziekte is te behandelen met een aantal antibiotica, waarvan metronidazol en vancomycine (de vorm die oraal moet worden ingenomen) en sinds kort fidaxomicine het meest gebruikt worden. Deze antibiotica leiden tot een geleidelijke verbetering van de klachten, maar helaas is de sporevorm niet gevoelig voor antibiotica. Na de behandeling kunnen de sporen weer ontkiemen en opnieuw klachten geven, mede omdat de darmbacteriën verder zijn aangetast door deze laatste antibiotica. Zo kan de ziekte steeds weer terugkomen, soms vele keren achter elkaar. Dit leidt tot ernstige verzwakking van de patiënt en eiwitgebrek, omdat deze zieke darm eiwit lekt.

Diagnose

De diagnose is te stellen door de toxines of de toxineproducerende *C. difficile*-stam in de ontlasting aan te tonen. Niet alle methoden zijn even goed zijn in het aantonen van de infectie en het is niet altijd mogelijk onderscheid te maken tussen infectie en kolonisatie, waarbij *C. difficile* wel aanwezig is, maar de klachten veroorzaakt worden door iets anders.

Verspreiding

De bacterie werd tot voor kort gezien als een typische ziekenhuisbacterie, omdat patiënten tijdens hun ziekenhuisopname een *C. difficile*-infectie kunnen krijgen. De verklaring daarvoor kan zijn dat de meest vatbare patiënten, ouderen met chronische ziekten die behandeld worden of zijn met antibiotica, in het ziekenhuis geconcentreerd zijn. De bacterie kan daar efficiënt worden overgedragen via voorwerpen en de handen van ziekenhuispersoneel, zich vermenigvuldigen en in sporevorm overleven. Daarbij komt nog dat de sporen niet gedood worden door veel gebruikte desinfectiemiddelen, zoals alcohol. Vanaf de ingang van het nieuwe millennium werden ziekenhuizen in Canada, gevolgd door de VS, het Verenigd Koninkrijk, Nederland en veel andere Europese landen, getroffen door uitbraken van *C. difficile*-infectie met een hoge sterfte en veel recidieven. Deze uitbraken bleken veroorzaakt door een specifieke *C. difficile*-stam, volgens de meest gangbare typeringsmethode aangeduid als PCR-ribotype 027. Door deze ontwikkeling ontstond er opnieuw aandacht voor *C. difficile*-infectie en heeft het onderzoek ernaar een grote vlucht genomen. Inmiddels worden gemiddeld zo'n 15 wetenschappelijke artikelen per week gepubliceerd over het onderwerp. Het idee dat *C. difficile*-infectie voornamelijk in het ziekenhuis wordt opgelopen, wordt tegenwoordig in twijfel getrokken. Dit komt onder meer omdat typeringsstudies vaak geen verband tussen verschillende gevallen in het ziekenhuis kunnen leggen, tenzij er sprake is van een duidelijke ziekenhuisuitbraak.

Behandeling

Er is nog geen behandeling voor *C. difficile*-infectie die de kans op recidieven tot nul reduceert. Antibiotica hebben het inherente nadeel dat ze de darmbacteriën ook aantasten en daarmee de kans op een recidief vergroten. Ook na behandeling met het nieuwe middel fidaxomicine, dat relatief selectief *C. difficile* doodt, treden recidieven op. Er is daarom gezocht naar niet-antibiotische behandelingen. Toxinebindende harsen bleken onvoldoende werkzaam. Er is nog geen vaccin beschikbaar tegen *C. difficile*-infectie, hoewel er studies met een experimenteel vaccin worden uitgevoerd. Een andere optie is het toedienen van antistoffen, die de toxines van de bacterie onschadelijk maken of op andere wijze het functioneren van de bacterie hinderen. Men onderscheidt verschillende vormen van deze zogenaamde passieve immunotherapie. Ten eerste is er een gradatie in de selectiviteit van de antistoffen. Er zijn ongeselecteerde antistoffen van bloedbankdonoren (antistoffen tegen *C. difficile*-toxinen komen in de algemene bevolking voor). Meer selectief zijn mengsels van antistoffen afkomstig van dieren die gevaccineerd zijn tegen *C. difficile* en/of de toxinen. Het meest specifiek zijn zogenaamde monoclonale antistoffen, die in een laboratorium geproduceerd worden. Ten tweede kan de toedieningsweg verschillen: intraveneus of oraal. Een combinatie van twee intraveneus toegediende monoclonale

antistoffen tegen de toxines TcdA en TcdB bleek de kans op recidieven na antibiotische behandeling te verminderen. De wijze van analyse van de studie was alleen twijfelachtig, omdat de studie in eerste instantie was opgezet om aan te tonen dat de ziekte minder ernstig zou verlopen na toevoeging van deze antistoffen, maar de onderzoekers er later voor kozen te kijken naar recidieven bij die patiënten die diarreevrij waren geworden. Op die manier is er een selectie aangebracht die in het voordeel van de antistoffen kan werken, omdat patiënten die ondanks de antistoffen diarree hielden, niet werden meegenomen in de analyse. Naast immunotherapie is behandeling met micro-organismen geprobeerd. Bekend zijn de probiotica, de 'goede' bacteriën en gisten die tegenwoordig aan allerlei zuivelproducten worden toegevoegd. Deze hebben niet overtuigend aangetoond dat ze de uitkomst van *C. difficile*-infectie kunnen beïnvloeden. Een behandeling waarvoor het bewijs wel heel sterk is, is het toedienen van een heel ecosysteem van micro-organismen in de vorm van feces van een gezonde donor, de zogenaamde fecestransplantatie. Deze lijkt ook effectief bij patiënten die meerdere recidieven hebben gehad, een patiëntencategorie waarvoor tot nu toe geen enkele andere behandelwijze bewezen effectief is. Tot slot wordt onderzocht of toediening van *C. difficile*-stammen die géén toxines maken, van nut kan zijn om *C. difficile*-infectie te voorkomen of behandelen door competitie met de toxineproducerende stammen aan te gaan.

Onbeantwoorde vragen

Er blijven veel onbeantwoorde vragen in het onderzoeksveld van *C. difficile*-infectie. Zo is het bijvoorbeeld niet duidelijk waar nieuwe stammen vandaan komen die zich vervolgens verspreiden en wat hun opkomst uitlokt en stimuleert. Verder is het niet goed te voorspellen welke patiënten na behandeling een recidief zullen krijgen. Dit is belangrijk, omdat de keuze van therapie kan afhangen van de inschatting of er een grote kans is op een recidief. Tot slot is er nog geen therapie die de kans op recidieven tot nul reduceert. De onderzoeken beschreven in dit proefschrift richten zich op diverse aspecten van *C. difficile*-infectie die gecompliceerd wordt door een ernstig zieke darm of door recidieven. Allereerst gaat het om de verspreiding van *C. difficile*-stammen die met zo'n ernstig of recidiverend beloop geassocieerd zijn, vervolgens over het voorspellen van zo'n beloop en tot slot over hoe behandeling de kans op zo'n beloop zou kunnen minimaliseren.

Hoofdstuk 1

Hierin worden twee gevallen van *C. difficile*-infectie die optraden buiten het ziekenhuis beschreven. Het eerste geval lijkt ook echt opgelopen buiten het ziekenhuis door een patiënte die niet aan het klassieke risicoprofiel voldoet, het tweede blijkt waarschijnlijk wel opgelopen in het ziekenhuis. Naar aanleiding van deze gevallen wordt een overzicht gegeven van de wetenschappelijke literatuur over *C. difficile*-infectie

opgelopen buiten het ziekenhuis. Daaruit blijkt dat er grote verschillen in definitie van buiten het ziekenhuis opgelopen *C. difficile*-infectie zijn, maar dat er vaker patiënten worden beschreven zonder de gebruikelijke risicofactoren recent antibioticum-gebruik, hoge leeftijd, chronische ziekte en frequent contact met zorginstellingen.

Hoofdstuk 2

Dit hoofdstuk beschrijft een studie waarbij patiënten die zich meldden in de huisarts-praktijk met diarree, werden getest op *C. difficile*-infectie, ongeacht de vraagstelling van de huisarts. Er werd bij 1,5% van de patiënten *C. difficile*-toxine gevonden. Drieëntwintig procent van deze patiënten was niet opgenomen geweest in een zorginstelling in het jaar daarvoor en had evenmin antibiotica gebruikt in het afgelopen halve jaar. Opvallend was dat de epidemische stam PCR-ribotype 027 niet werd aangetroffen, ondanks het feit dat in de drie regio's waar de studie werd uitgevoerd, Haarlem, Utrecht en Zwolle, ziekenhuisuitbraken waren geweest met deze stam. Ook werden verschillende zeldzame en niet eerder getypeerde stammen gevonden, die meestal niet gevonden worden in ziekenhuizen. Een zwakte van de studie was dat andere verwekkers van diarree niet stelselmatig werden uitgesloten, er werd alleen gekeken naar de ziekteverwekkers waarvoor de huisarts een test had aangevraagd. Onderzoek naar een andere verwekker was nooit positief als *C. difficile*-toxine werd aangetroffen. Onze conclusie was dat buiten het ziekenhuis veel verschillende *C. difficile*-stammen circuleren. Deze stammen koloniseren de menselijke darm onder bepaalde omstandigheden, zoals na aantasting van de darmbacteriën door antibiotica, en veroorzaken eventueel ziekte. Waarschijnlijk gaat een deel van deze ziekte-episoden vanzelf voorbij zonder behandeling, zeker als de diarree duidelijk uitgelokt is door antibiotica en deze antibiotica vervolgens weer gestopt worden.

Hoofdstuk 3

Dit hoofdstuk beschrijft een studie naar dragerschap van *C. difficile* bij patiënten met cystic fibrosis, ook wel bekend als taaislijmziekte. Deze genetische ziekte leidt tot de vorming van veel dikker slijm in de luchtwegen dan bij gezonde mensen. Hierdoor worden bacteriën moeilijk verwijderd door de trilharen van de luchtwegen. Het gevolg is het herhaaldelijk optreden van bronchitis en longontsteking, waardoor de luchtwegen beschadigd raken. Logischerwijs worden deze patiënten vaak met antibiotica behandeld en komen ze regelmatig in ziekenhuizen. Je zou verwachten dat *C. difficile*-infectie veel voorkomt bij deze patiënten, maar studies uit de jaren '80 tonen aan dat ze vaak drager zijn van *C. difficile*, maar geen klachten hebben. We wilden deze oude waarneming bevestigen nu *C. difficile*-infectie frequenter voorkomt en er epidemische stammen zijn. We hebben hiervoor aan alle cystic fibrosis-patiënten van het Erasmus Medisch Centrum in Rotterdam gevraagd ontlasting in te leveren.

Ongeveer de helft van hen (55 patiënten) leverde ontlasting in. We vonden dat 47% van de patiënten drager was van *C. difficile*. Geen van deze patiënten had diarree. Opvallenderwijs bleek 77% van de gekweekte stammen geen toxine te produceren. Dit is een veel hoger percentage dan we aantroffen bij een controlegroep van patiënten opgenomen op een afdeling Interne Geneeskunde van het Ziekenhuis Bronovo in Den Haag. Dragerschap bij cystic fibrosis-patiënten was verrassenderwijs geassocieerd met een lagere leeftijd en - niet zo verrassend - met ernstiger cystic fibrosis. Er werden veel verschillende, deels zeldzame stammen gevonden, wat suggereert dat de patiënten de stammen niet van elkaar hadden gekregen en evenmin in het ziekenhuis hadden opgelopen. Ook deze bevinding sluit aan bij de conclusie van het vorige hoofdstuk dat er veel verschillende *C. difficile*-stammen buiten het ziekenhuis circuleren. Verder is het de vraag waarom er zoveel niet-toxine-producerende stammen worden gevonden bij patiënten met cystic fibrosis. Mogelijk circuleren deze stammen ook in de gemeenschap, maar kunnen ze de darmen van mensen zonder cystic fibrosis moeilijk koloniseren. De andere samenstelling van het darmslijm en/of de darmbacteriën bij cystic fibrosis-patiënten leidt er misschien toe, dat niet-toxineproducerende stammen hun darmen wel kunnen koloniseren. Ook hebben toxineproducerende stammen misschien geen voordeel ten opzichte van niet-toxineproducerende stammen bij de kolonisatie van de darm van cystic fibrosis-patiënten. Hiervoor zijn meerdere hypothesen te bedenken. De toxines dringen mogelijk moeilijk door de darmslijmlaag van cystic fibrosis-patiënten, of de toxines kunnen minder schade berokkenen, doordat het eiwit dat afwijkend is bij cystic fibrosis-patiënten een rol speelt bij de werking van de toxines. Dit laatste zou ook kunnen verklaren waarom cystic fibrosis-patiënten zo weinig klachten van toxine-producerende *C. difficile*-stammen hebben. Kolonisatie met niet-toxineproduceren-de *C. difficile*-stammen zou de cystic fibrosis-patiënten ook kunnen beschermen tegen ziekte door hun toxineproducerende verwanten. Zoals eerder vermeld wordt er zelfs onderzoek gedaan met niet-toxineproducerende stammen voor preventie en behandeling van *C. difficile*-infectie. Hierbij is een opvallende eerste bevinding dat kolonisatie met niet-toxineproducerende *C. difficile* alleen bereikt kan worden bij gezonde vrijwilligers als ze eerst 5 dagen met vancomycine behandeld worden en dan 14 dagen niet-toxineproducerende *C. difficile* toegediend krijgen.

Hoofdstuk 4

Om beter in kaart te brengen welke *C. difficile*-stammen in Europese ziekenhuizen voorkomen na de opkomst van de epidemische stam PCR-ribotype 027, en of infecties door deze stammen leiden tot complicaties en recidieven, hebben we een netwerk opgezet van 106 laboratoria, verbonden aan Europese ziekenhuizen. Het vóórkomen van *C. difficile*-infectie in de ziekenhuizen bleek sterk te variëren. De ziekte kwam het meest voor in ziekenhuizen in noordelijke landen, waar het antibioticumgebruik in het

algemeen laag is. Misschien zijn er veel gevallen gemist in de Zuid-Europese ziekenhuizen. Als er echt een verschil is in het optreden van *C. difficile*-infectie tussen Noord- en Zuid-Europa, zijn mogelijke verklaringen hiervoor een verschil in de ernst van chronische ziekten van de opgenomen patiënten (misschien is de drempel tot opname hoger in landen met een goed georganiseerde eerstelijnszorg) of verschil in de efficiëntie van overdracht (misschien draagt een kouder klimaat bij aan efficiëntere overdracht, bijvoorbeeld doordat sporen langer overleven of mensen dichter op elkaar leven). PCR-ribotype 027 bleek niet het meest voorkomende type in de deelnemende Europese ziekenhuizen – het kwam op de zesde plaats. De top drie bestond uit PCR-ribotypes 014/020, 001 en 078. Het laatste type is interessant, omdat het eigenschappen deelt met PCR-ribotype 027, hoewel het wat minder schadelijk lijkt. Het werd in een kleinere Europese studie in 2005 nog vrijwel niet gevonden. Ook in Nederland is dit type inmiddels een van de meest voorkomende types geworden. Verder blijkt het ook bij boerderijdieren veel voor te komen. Dit roept de vraag op of het ribotype via vlees overgedragen wordt. Er zijn echter nooit uitbraken herleid tot een voedselbron.

Verder viel op dat na drie maanden 22% van de patiënten overleden was, wat illustreert dat *C. difficile*-infectie in ziekenhuizen nog steeds een ziekte is van oudere mensen met chronische ziekten. De behandelde artsen schatten in, dat *C. difficile* medeverantwoordelijk was voor een derde van deze sterfgevallen en zelfs direct verantwoordelijk voor 7%. Infecties door PCR-ribotypes 027, 015 en 018 hadden een ernstiger beloop dan de overige ribotypen.

Hoofdstuk 5

Zoals vermeld in de inleiding, is het niet goed te voorspellen hoe het beloop van een *C. difficile*-infectie zal zijn en kan het verwachte beloop wel relevant zijn voor de keuze van behandeling. Er is geprobeerd goede voorspellers te vinden en er zijn diverse bevindingen, vooral bloedbepalingen, in verband gebracht met complicaties of recidieven. Het nut van die bloedbepalingen in relatie tot elkaar is niet duidelijk en de verbanden zijn gevonden in kleine studies. We hebben de databases van twee grote geneesmiddelenstudies voor de behandeling van *C. difficile*-infectie gebruikt om drie voorspellers van een ongunstig beloop te onderzoeken. Dit waren lichaamstemperatuur, aantal witte bloedcellen in het bloed en een bloedbepaling die de nierfunctie weerspiegelt (creatinine). De uitkomsten waren de duur van de diarree en het optreden van recidieven na verdwijnen van diarree. Vooral een slechte nierfunctie bij diagnose bleek samen te hangen met het voortbestaan van diarree en het optreden van recidieven. Verder bleek in deze studie dat de waarden van witte bloedcellen en nierfunctie per patiënt sterk varieerden rond het stellen van de diagnose *C. difficile*-infectie.

Hoofdstuk 6

Een belangrijke andere vraag bij *C. difficile*-infectie is hoe groot de rol van het immuunsysteem is bij het beloop van de infectie, met name de antistoffen. Deze antistoffen, die worden geproduceerd door bepaalde witte bloedcellen, zijn heel specifiek gericht tegen een ziekteverwekker (bacterie, virus, schimmel, eencellige of worm) of een toxine. Ze kunnen de ziekteverwekker markeren om het opruimen van de ziekteverwekker door het immuunsysteem te vergemakkelijken, de ziekteverwekker hinderen in zijn functioneren, of toxines onschadelijk maken. Antistoffen worden niet van nature gemaakt - het afweersysteem moet namelijk 'leren' antistoffen te maken tegen de ziekteverwekkers of toxines waarmee het op dat moment geconfronteerd wordt. Voor de meeste infecties geldt, dat als eenmaal een antistofreactie op gang gekomen is, het afweersysteem vervolgens geheugen opbouwt en er bij een volgende confrontatie met deze ziekteverwekker sneller antistoffen worden aangemaakt dan bij de eerste confrontatie. Het ligt voor de hand bij *C. difficile*-infectie te kijken naar antistoffen, omdat de ziekteverschijnselen het gevolg zijn van toxines en toxines bij uitstek een goed doelwit zijn voor antistoffen.

Voor de studie beschreven in dit hoofdstuk hebben we gebruik gemaakt van opgeslagen serummonsters (bloed ontdaan van cellen en stollingsfactoren) van patiënten met *C. difficile*-infectie die hadden deelgenomen aan de studie die beschreven staat in het volgende hoofdstuk. We wilden de associatie tussen antistoffen en het optreden van recidieven onderzoeken. Die associatie is interessant voor het oorzakelijk denken over *C. difficile*-infectie (Welke rol speelt de antistofreactie bij het optreden van recidieven van *C. difficile*-infectie?) en het voorspellen van recidieven. Patiënten met hoge serum-concentraties van antistoffen tegen de toxines TcdA en TcdB bleken meestal geen recidieven te krijgen. Dit gold niet voor antistoffen tegen eiwitten op het oppervlak van *C. difficile.* Deze bevindingen ondersteunen de hypothese dat antistoffen een rol spelen bij het beloop van *C. difficile*-infectie. Ze vormen echter niet de hele verklaring voor het optreden van recidieven. Waarschijnlijk zijn andere factoren belangrijker, zoals de diversiteit en het aantal van de darmbacteriën. Verder was opvallend dat de concentraties antistoffen tijdens de infectie vaak daalden, wat merkwaardig is voor een antistofreactie tijdens en direct na een opgelopen infectie. Een mogelijke verklaring is dat er eiwitten, waaronder antistoffen, uit de zieke darm lekken naar de ontlasting en de antistoffen op die manier verdwijnen. Een nadeel van deze studie was het feit dat de patiënten allemaal een experimenteel middel namen, dat antistoffen tegen *C. difficile* afkomstig van koeien bevatte. De metingen van de menselijke antistoffen werden overigens niet verstoord door de aanwezigheid van de dierlijke antistoffen. Dit experimentele middel was bedoeld om recidieven te voorkomen. Daardoor zou de sterkte van de associatie tussen antistoffen en het optreden van recidieven kunnen zijn afgenomen.

Hoofdstuk 7

Dit hoofdstuk beschrijft de studie die in de vorige paragraaf genoemd werd. Patiënten die zojuist tenminste 10 dagen succesvol behandeld waren voor *C. difficile*-infectie met antibiotica, namen een experimenteel product in om recidieven te voorkomen. Dit product was gemaakt van melk van koeien die gevaccineerd waren met gedode *C. difficile* en onschadelijk gemaakte *C. difficile*-toxines. Hierdoor bevatte de melk een hoge concentratie antistoffen tegen de bacterie en de toxines. De eiwitfractie van de wei van deze melk was vervolgens verwerkt tot een poeder, dat opgelost moest worden met water en drie maal per dag ingenomen. Bij proefdieren bleek het product effectief te zijn. In vergelijking met bekende recidiefpercentages hadden de studie-patiënten weinig recidieven: deze traden op bij 11 van de 109 ziekte-episoden (10%). Dit is echter geen bewijs dat het product werkt, want daarvoor zou een studie noodzakelijk zijn met een placebo-arm, waarbij het lot bepaalt of een patiënt placebo of het wei-eiwit krijgt en patiënt en onderzoekers tijdens de studie niet weten welk van beide de patiënt krijgt. Mits er voldoende patiënten in de studie zitten, is dit de beste garantie dat een verschil tussen beide studie-armen verklaard kan worden door het wei-eiwit en niet door verschillen in ziekte-ernst of andere factoren die moeilijk meetbaar zijn.

Hoofdstukken 8 en 9

Hierin staan de eerste en tweede richtlijn voor behandeling van *C. difficile*-infectie, die we hebben opgesteld in opdracht van de European Society for Clinical Microbiology and Infectious Diseases. We doen hierin aanbevelingen voor keuze van therapie op grond van de beschikbare wetenschappelijke literatuur.

Met de studies beschreven in de voorgaande hoofdstukken hopen we de kennis over *C. difficile*-infectie enigszins vergroot te hebben. Nieuwe bevindingen waren onder meer dat *C. difficile*-infectie in de Nederlandse huisartsenpraktijk voorkomt, soms zonder de gebruikelijke risicofactoren. Verder blijken Nederlandse patiënten met cystic fibrosis vaak asymptomatisch gekoloniseerd met *C. difficile*-stammen, die overwegend geen toxine produceren. Onze studie in Europese ziekenhuizen toonde aan dat de epidemische stam PCR-ribotype 027 niet de meest dominante stam geworden is. Tot slot bleken een slechte nierfunctie en lage serumconcentraties van antistoffen tegen *C. difficile*-toxines geassocieerd met een later recidief van *C. difficile*-infectie.

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Curriculum vitae

Martijn Philippe Bauer werd op 8 mei 1974 geboren in Leiden. Hij behaalde zijn eindexamen in 1992 aan het Erasmiaans Gymnasium in Rotterdam. Vervolgens studeerde hij geneeskunde in Groningen. Deze studie rondde hij af met een onderzoeksstage in het Kabale Hospital in Oeganda. Hij doorliep zijn co-assistentschappen in het Medisch Spectrum Twente, Enschede, en het Diakonessenhuis Utrecht en behaalde zijn artsexamen cum laude in 1999. Na een jaar als arts-niet-in-opleiding in het Leids Universitair Medisch Centrum begon hij in 2001 aan zijn opleiding tot internist in hetzelfde ziekenhuis met prof. dr. A.E. Meinders als opleider, gevolgd door prof. dr. J.A. Romijn. Eén jaar van zijn opleiding bracht hij door in het Ziekenhuis Bronovo met dr. J.W. van **β** Wout als opleider. In 2006 rondde hij zijn opleiding tot internist af en in 2007 werd hij geregistreerd als internist-infectioloog en in 2012 ook als internist acute geneeskunde. In 2007 begon hij aan het promotie-onderzoek bij de afdeling infectieziekten van het Leids Universitair Medisch Centrum onder leiding van prof. dr. J.T. van Dissel en prof. dr. E.J. Kuijper, waarvan de resultaten in dit proefschrift beschreven worden. Vanaf diezelfde tijd werkt hij als internist-infectioloog in het Leids Universitair Medisch Centrum en vanaf 2010 ook als internist op de Spoedeisende Hulp en de Acute Opname-afdeling. Gedurende zijn studie, co-assistentschappen, opleiding en de periode daarna ontplooide hij verschillende nevenactiviteiten, onder meer als lid van het bestuur van de Stichting Werkgroep Antibioticabeleid en het bestuur van de Trainees Association van de European Society for Clinical Microbiology and Infectious Diseases.