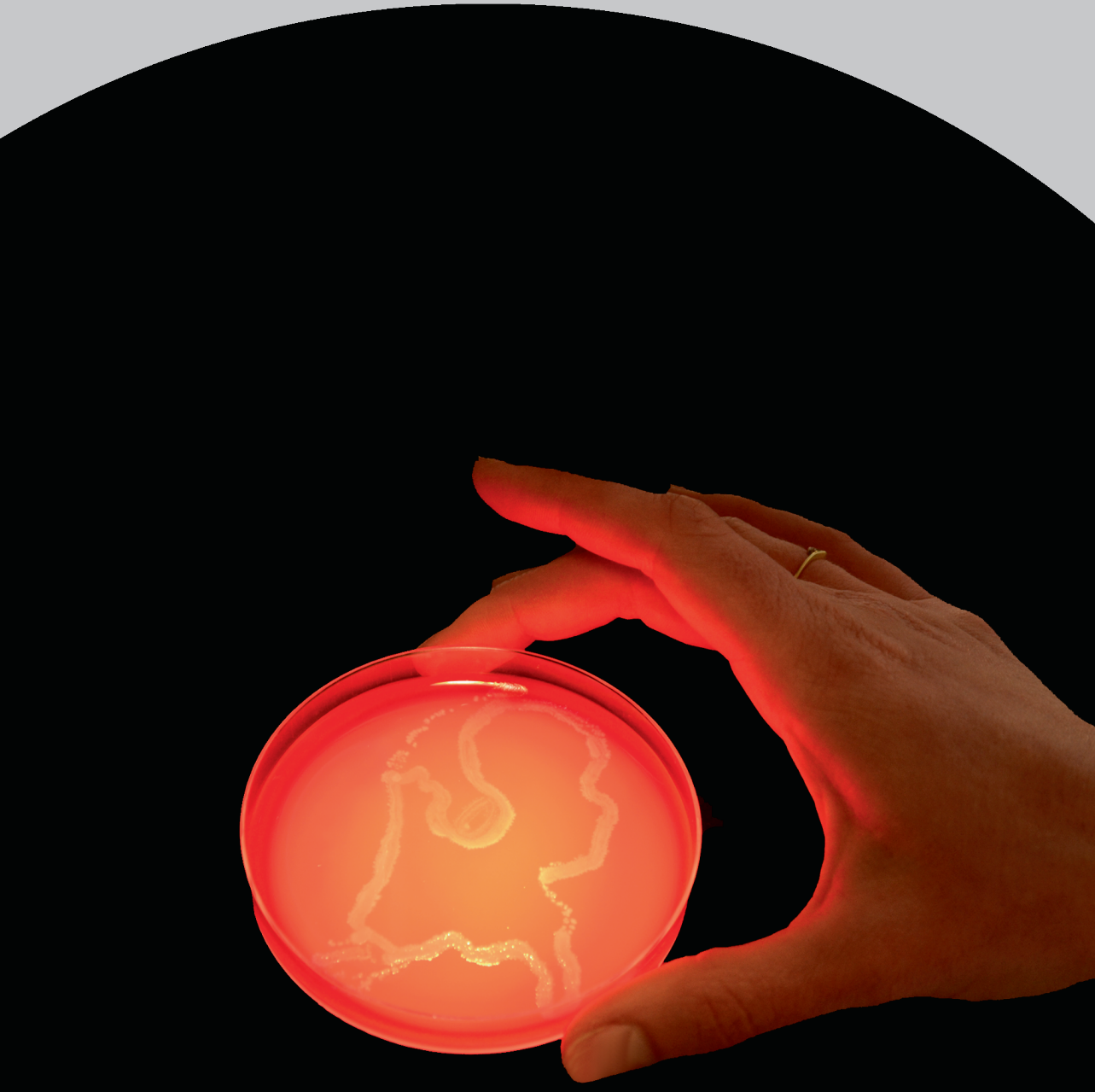

Risk factors, course and outcome of *Clostridium difficile* infections

Marjolein Hensgens

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Cover Photo: by Marlous Gaarlandt – Nacken and Céline Harmanus
Representing a blood agar plate with a *Clostridium difficile* type 078 isolate, growing according to the geographical map of the Netherlands
Print & lay-out: Gildeprint Drukkerijen – Enschede, the Netherlands
ISBN/EAN: 978-94-6108-512-2

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Proefschrift

ter verkrijging van de graad van Doctor
aan de Universiteit Leiden
op gezag van Rector Magnificus prof. mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 15 oktober 2013 klokke 16.15 uur

door

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geboren 1 maart 1984
te Heerlen

Promotiecommissie

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Introduction

Chapter 1

General introduction and outline of the thesis

General introduction

This thesis focuses on patients with diarrhoea due to the gastrointestinal bacterium *Clostridium difficile*. Hospitalized patients with a *Clostridium difficile* infection (CDI) often have a history of antibiotic use and underlying diseases. However, many hospitalized patients without CDI also fit this profile. In general practitioners' practice, a third of the CDI patients lack risk factors such as recent antibiotic use and multiple comorbidities. Therefore, additional factors for recognition of patients with CDI are needed.

In this thesis, we have aimed at identifying patients at risk for CDI. In addition, we have aimed at recognizing factors associated with a complicated course and outcome of the disease, as we currently do not know which patients are at risk of deterioration and failure of therapy.

Introduction

Twenty percent (12-32%) of all hospitalized patients experience diarrhoea during their stay in the hospital[1-3]. Often this diarrhoea is noninfectious and caused by enteral feeding, underlying diseases or medication, whereas 10-30% has an infectious origin[4]. By far, the most frequent infectious cause of nosocomial diarrhoea is *Clostridium difficile*. Other bacterial pathogens are found in as little as 0.5% of the stool cultures of hospitalized patients[5]. The most important viral pathogen, norovirus, has a typical clinical presentation, is season dependent and mainly associated with outbreaks[4]. Infections caused by *C. difficile* are nowadays notorious for their high morbidity and mortality risk. Mild diarrhoea may be the only symptom of a *Clostridium difficile* infection (CDI) but bloody diarrhoea accompanied by severe inflammation of the colon, so called 'pseudomembraneous colitis', can be present[6]. Symptoms mainly occur in elderly patients or patients who received antibiotic therapy. During outbreaks of CDI, 15% of the infected patients were reported to die in the first 30 days after diagnosis, as a consequence of the infection[7, 8]. Several studies translated this large impact of CDI to an economic burden, concluding that CDI probably results in over a billion dollar excess costs in health care facilities in the United States[9, 10].

The bacterium

C. difficile is part of the genus *Clostridium*, which is formed by approximately hundred rod-shaped, anaerobic and Gram-positive species. Among these, five toxin

producing species are particularly associated with human disease: *C. botulinum* (botulism; paralytic disease), *C. perfringens* (disease varying from food poisoning to myonecrosis), *C. tetani* (tetanus), *C. sordellii* (myonecrosis) and *C. difficile* (colitis). The name *C. difficile* originates from the difficulty with which this organism could be cultured: it grew slower than the other *Clostridia*[11].

All five notorious *Clostridia* produce toxins that are responsible for disease. Although all are named 'toxins', there is a wide variety in their biological target (e.g. neurotoxins or enterotoxins). *C. difficile* produces enterotoxin A and B. Both toxins enter the cells of the gut by endocytosis, whereafter they undergo structural changes that enable them to inactivate enzymes of the small GTPases family (e.g. Rho and Rac) in the cytosol[12-14]. As these enzymes are involved in many signaling pathways, inactivation leads to inhibited enteric cell division and trafficking and changes in the cell morphology, subsequently leading to cell death. Although under debate, both toxins individually seem to be able to cause disease[15, 16]. Virulent *C. difficile* strains are often associated with an increased toxin production, however, recent reports suggest that hyperproduction of toxin is not intrinsically associated with severe disease[17]. Mutations in the *tcdC* gene were regarded as the cause of increased toxin production[18]. Two relatively new publications, however, question the function of *tcdC* as a negative regulator of toxin production[19, 20]. At a different location in the bacterial genome, genes for a third toxin are situated: binary toxin (encoded by two genes *cdtA* and *cdtB*)[21]. Though this toxin is probably insufficient to cause disease on its own, it seems to enhance adherence and colonization of *C. difficile*[21, 22]. Binary toxin induces the development of long protrusions at the surface of intestinal epithelial cells which are hypothesized to form a dense web that embed bacteria such as *C. difficile* and thereby promote colonization[21]. Isolates that cause severe human CDI are often binary toxin positive, but there is debate if binary toxin is the cause of this severe clinical presentation[23, 24]. Several other virulence factors enable *C. difficile* to cause disease, such as the ability of *C. difficile* to produce spores, a dormant form in which nutrients are unnecessary. Commonly used disinfectants, antibiotics and gastric acid are harmless to spores[25]. Therefore, spores enable *C. difficile* to survive for a long time in aerobic environments, outside patients. In hospitals, *C. difficile* spores can survive for many months[26]. Consequently, *C. difficile* can be transmitted by both direct contact with a patient and contact with the environment around the patient e.g. the bedside table, chair, rectal thermometer, telephone and a blood pressure cuff[27, 28]. Other bacterial factors such as certain surface layer proteins (SLPs) or increased antibiotic resistance might also contribute

to the virulence and spread of *C. difficile*[29, 30]: SLPs have been hypothesized to be involved in colonization or evasion of the immunesystem, while antibiotic resistance might cause increased spread and infection among patients with antibiotic use. All aforementioned bacterial characteristics were hypothesized to increase virulence of *C. difficile*[31]. However, the exact molecular basis for the increased pathogenicity of some *C. difficile* strains remains to be elucidated.

To study the epidemiology of CDI, various molecular typing methods are available. Currently, the most frequently used method in Europe is PCR ribotyping. This method considers the variability of the intergenic spacer region between the 16S and 23S rDNA, which may be present in multiple copies. After PCR amplification, the amplicons form a banding pattern when separated by gel electrophoresis. This banding pattern is referred to as PCR ribotype[32]. In 2012, more than 400 PCR ribotypes were known, but the discriminatory power is still insufficient. Using PCR ribotyping, an increase in prevalence of a type can therefore provide a clue for an outbreak but does not necessarily prove clonal spread of one strain[33]. Recently, PCR ribotyping was adapted to capillary gel electrophoresis, which improved the resolution of the banding patterns and therefore the discriminatory power[34]. To discriminate strains within a single PCR ribotype, multilocus variable-number tandem repeat analysis (MLVA) was developed[35]. This molecular typing method is currently used to study outbreak situations and to determine routes of transmission between patients and hospitals[36]. However, the most promising method to overcome problems with discriminatory power in future is examination of the entire genome of *C. difficile* using e.g. 'whole genome single nucleotide polymorphism typing'[33]. The first study using this sequencing method showed a high discriminative ability in 15 patients with healthcare-associated *C. difficile*, resulting in different conclusions regarding a recent 'outbreak'[37]. As not all patients with the same PCR ribotype had an identical *C. difficile* strain, not all infected patients were part of the outbreak. Consequently, patient management and infection control practice during the outbreak altered. Other challenges for whole genome sequencing are the discovery of patterns of evolution and the prediction of emergence of disease[38]. A recent analysis of a global collection of 151 isolates with a single *C. difficile* PCR ribotype (type 027), revealed that two epidemic lineages emerged simultaneously, subsequently causing problems around the world[39]. Although data interpretation of whole genome single nucleotide polymorphism typing is complex and costs are high, this approach will likely become the preferred typing method over the coming years as easy-to-use software is being developed and costs are dropping fast[33].

Occurrence of *C. difficile* in human disease

In 1935, *C. difficile* was discovered as a colonizing organism in the gastrointestinal tract of children[40]. Later on, *C. difficile* was identified in the environment, including soil and water, and in the intestines of numerous animal species[41, 42]. Many years however passed until *C. difficile* was recognized as a major human pathogen. In 1978, *C. difficile* was first identified as the causative agent of antibiotic associated diarrhoea[11, 43, 44]. After this discovery, *C. difficile* was described as a potential dangerous pathogen that was fortunately largely controlled[43]. This statement remained genuine up to 2004, when major outbreaks were reported all over the western world.

Canada and the United Stated[45, 46] were first to report outbreaks with extremely high incidences and a more severe disease course. During a 13-year period (1991-2003) incidences of *C. difficile* in Canada quadrupled[45]. The case-fatality risk more than doubled over time: in 2003 18% of the patients with CDI died within 30 days after diagnosis[45]. In Europe, similar experiences were reported in England, the Netherlands, Belgium, France and Germany[47, 48]. The major cause of most outbreaks was *C. difficile* PCR ribotype 027[7, 30]; a more virulent *C. difficile* PCR ribotype. This type was associated with excess toxin production and sporulation and the presence of binary toxin[49, 50]. Additionally, newer isolates of type 027 were highly resistant to fluoroquinolones, in contrast to type 027 isolates that caused disease before 2001[30]. Increased use of these antibiotics might therefore have contributed to its rapid spread[51].

In response to these outbreaks, the urgency to recognize type 027 grew[52, 53] and consequently, the number of countries in which type 027 was identified increased. In the Netherlands, the first large outbreak due to type 027 was detected in July 2005[54]. Incidences in the hospital of Harderwijk increased from 4 to 83 per 10,000 admissions; a second hospital reached incidences above 80 per 10,000 admissions a month later, probably following the transfer of a patient. As was seen in other outbreaks, type 027 was associated with the use of fluoroquinolones[55, 56] and high mortality risks[55]. Soon after the first outbreak, type 027 was detected in numerous Dutch healthcare facilities and national protocols to recognize and combat outbreaks were developed[55]. Additionally, a national reference laboratory was initiated to type and characterize *C. difficile* isolates that were involved in outbreaks or caused severe symptoms of CDI.

New era of *C. difficile* infections

In Chapter 2 of this thesis, the epidemiology of CDI in the years following the outbreaks is described[57]. In short, the incidence in the Netherlands became stable at 14-18 per 10,000 admissions and type 027 became less prevalent[57-60]. Similarly, in England the incidence of CDI due to type 027 decreased and the number of death certificates that mentioned *C. difficile* lowered[61]. Besides England and the Netherlands, other (parts of) countries entered an endemic state[62] and a European-wide study in 34 countries reported that type 027 was no longer among the 10 most frequently found PCR ribotypes in hospitals[63]. As we are currently (2013) past the era of major outbreaks of nosocomial CDI in the Netherlands, this new era in which CDI is stably present provides a good situation to study risk factors and outcome of CDI without the selection of a specific *C. difficile* strain or the selection of a specific study population as occurs during outbreaks.

Risk factors for nosocomial infection

Elderly patients and patients with underlying diseases or recent antibiotic use are at risk for CDI. Antibiotics are notorious due to their disruption of the normal gut flora that normally protects against colonization and infection by *C. difficile*. A resistant *C. difficile* isolate can immediately flourish, whereas other *C. difficile* isolates sporulate and wait until the antibiotic therapy is stopped, antibiotic levels in the gut have disappeared, but the flora is still disturbed (Figure 1).

One dosage of antibiotic therapy already makes the flora less diverse. Major changes in the flora disappear after weeks, whereas minor changes can be present up to two years after the antibiotic was used[65]. Together with the changes in microbiota, the risk for CDI is increased. It is however unknown how long the increased risk lasts after cessation of antibiotic therapy (Chapter 5). Almost all antibiotics have been associated with an increased risk for CDI although second, third and fourth generation cephalosporins, to which virtually all *C. difficile* strains are resistant, are often mentioned to carry the highest risk[66]. The virulent *C. difficile* type 027 is also highly resistant to fluoroquinolones, and epidemiological findings confirmed fluoroquinolones as a major risk factor for CDI due to this type[30,56]. Besides antibiotic use, several other risk factors for CDI have been described (Table 1). Older age, multiple underlying diseases and medication such as immunosuppressive and chemotherapeutic agents are most frequently mentioned. Additionally, exposure to other infected patients, so called 'infection pressure', increases the risk for CDI[67].

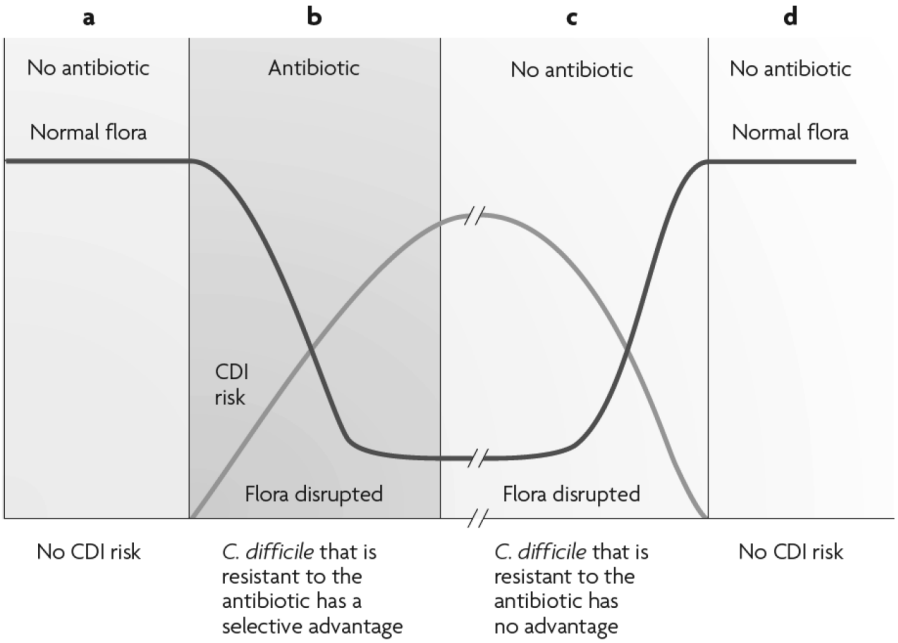

Figure 1. The effect of antibiotics on the normal gut flora and the risk of *Clostridium difficile* infection (CDI)[64].

A factor that decreases a patient's vulnerability to CDI is asymptomatic intestinal colonization by *C. difficile*[68]. Colonization with a toxin producing strain provokes a strong IgG response in some patients, preventing the development of disease[69]. Alternatively, colonization with a non-toxigenic strain may prevent disease by filling the intestinal niche[70]. A drawback of asymptomatic colonization with a toxin producing strain is that it exhibits a potential *C. difficile* reservoir that enables spread to other patients, causing colonization or disease.

The longer the hospitalization and the more *C. difficile* circulates in a healthcare facility, the higher the colonization risk. According to a recent Canadian study, 3% of the hospitalized patients became carriers of *C. difficile* during their hospitalization[72]. Colonization among healthy children and neonates is even more frequent. In a recent study by Rousseau et al., all 85 infants (0-3 years) that acquired *C. difficile* remained colonized for several months during the one-year follow-up[73]. As colonization in children often encompasses a toxigenic strain, children are also a potential reservoir of *C. difficile*.

Table 1. Risk factors for Clostridium difficile infections in hospitalized adults[67, 69, 71].

Risk factors
Age > 65 years
Antibiotic use
Prolonged duration of hospital stay / infection pressure
Underlying diseases Multiple comorbidities Inflammatory bowel diseases Immunodeficiency and HIV Chronic kidney disease requiring hemodialysis
Treatment other than antibiotics Chemotherapy Immunosuppressive agents Proton pump inhibitors Transplantation (solid organ or hematopoietic stem cell) Abdominal surgery
Host immune response No IgG response to toxins

Morbidity and mortality of CDI

Symptoms caused by CDI are stringent intestinal: extra-intestinal manifestations rarely occur. Commonly, CDI is defined as the presence of diarrhoea and a positive toxin test[74]; though cases without diarrhoea have been described[75, 76]. The severity of symptoms varies between mild diarrhoea and a pseudomembraneous colitis or toxic megacolon resulting in dehydration, shock or even death[6]. Complication rates, encompassing colectomy, admission to an intensive care unit and death due to CDI, have mainly been studied during outbreaks. Following an outbreak with a type 027 strain in Canada, complications due to CDI were present in 15% of 1,703 patients[7, 8]. When outbreaks occur in a population with severe underlying diseases, such as patients in an intensive care unit, complication risks may run up to 25%[77]. This high mortality risk among CDI patients, however, is not necessarily observed in the new era in which CDI is endemic (as currently in the Netherlands) or other outbreaks, because outbreaks involve a specific *C. difficile* strain as well as a specific population. In Chapter 7 and 10 of this thesis, we estimate the mortality among CDI patients in an endemic situation.

Patients cured from their initial CDI episode face another threat: a recurrence. Approximately 20% of the CDI patients suffer from recurrent symptoms in the first 60 days after initial cure (either a reinfection or relapse with the same strain)[78-81]. Although many studies focused on predictors of a recurrence[79, 82-84], only one validated prediction score currently exists[83]. This score includes four predictors: age >65 years, severe comorbidity, antibiotic use after discontinuation of CDI therapy

and a low host immune response to *C. difficile* toxins (low antitoxin A IgG). As one of the proposed mechanisms for recurrence is the persistent disturbance of the intestinal flora, treatments such as faecal transplantation or fidaxomicin show promising results in the treatment of recurrent CDI. These treatments maintain or even increase the diversity of the intestinal flora[85, 86]. The treatment of a first episode of CDI with metronidazole or vancomycin is focused on cure of the present infection and does not prevent additional changes of the gut flora, which makes these treatments less effective in recurrent CDI. More information on the treatment of CDI will be given in Chapter 11 of this thesis.

C. difficile in the community

In addition to the increase in incidence of CDI in hospitals, more and more CDI patients were detected outside healthcare facilities[87-89]. According to an analysis of all diagnoses made by general practitioners in the United Kingdom, the rates of CDI exponentially rose in time (Figure 2). Although the rise in incidence could be partly explained due to increased testing for *C. difficile,* a true increase is likely as the rise predated the introduction of mandatory reporting of CDI and coincided with the exponential increase of CDI in hospitals[87]. After 2004 the increase continued and the Health Protection Agency reported an incidence of 111 per 100,000 inhabitants in 2008. More recent data suggest a declining incidence of CDI within primary care organizations, with an incidence of 35 cases of CDI per 100,000 inhabitants in England in 2012[90].

It is estimated that 50% to 75% of all CDI currently occurs in the community[87, 91]. This percentage includes patients who develop CDI in the weeks following their discharge from a hospital and patients who acquire the infection in the community.

The interest towards CDI as an important pathogen of diarrhoea in the community, increased when patients without traditional risk factors developed CDI. Pregnant women, young individuals and patients without previous antibiotic use were increasingly diagnosed outside healthcare facilities[88, 92]. It remained unclear what made these patients susceptible to CDI (host factors) and where they acquired the pathogen (bacterial factors). As *C. difficile* frequently causes disease and colonization in animals, especially piglets and cattle, direct contact with these animals or transmission via their meat was suggested as a source of human CDI[93-96]. Additionally, colonized children, contaminated environment and food were hypothesized to transmit *C. difficile* to humans[92, 97, 98]. Several studies were performed to determine risk factors for CDI in the community, which are reviewed in Chapter

3 of this thesis. Additionally, we describe CDI in general practice in Chapter 6 and summarize the evidence for zoonotic transmission in Chapter 11.

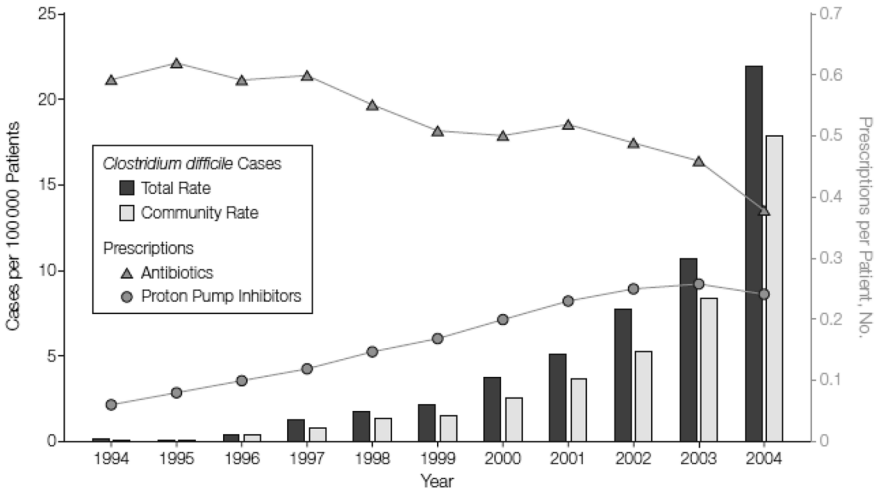

Figure 2. Rates of Clostridium difficile per 100 000 patients in the United Kingdom general practice research database[87].

Aims and outline of this thesis

Besides providing an overview of the current knowledge on the epidemiology of *C. difficile* infections, this thesis aims at answering two major questions in the clinical research of CDI: 'who is at risk for the infection' and 'what is the course and outcome of the infection'.

As we highlighted in the introduction, it is difficult to recognize CDI in general practice as diarrhoea is frequent and classical risk factors for CDI do not occur in all CDI patients. In contrast, risk factors for CDI are numerous among hospitalized patients, which also troubles recognition of CDI. In Part I of this thesis we aim at determining patients at risk for CDI in more detail and consequently enhance recognition of CDI. In Part II of this thesis, we aim at recognizing factors associated with a complicated course and outcome of the disease, as we currently do not know which patients are at risk of deterioration and failure of therapy. We end with a critical view on the content of this thesis and provide recommendations for further research.

Introduction

Chapter 1 is the current chapter in which we give a general introduction to CDI. We focus on CDI in hospitals.

Chapter 2 addresses the surveillance system in 13 hospitals in the Netherlands and displays the results of the national reference laboratory that was started soon after the first Dutch outbreaks occurred. Furthermore, this chapter describes the molecular epidemiology and incidence of CDI in the Netherlands.

Chapter 3 introduces us to CDI in the community by reviewing the available literature on the incidence of CDI and possible sources and routes of transmission of *C. difficile*.

Part I: Population at risk for a *Clostridium difficile* infection

Chapter 4 describes risk factors for CDI in an endemic setting. As most studies focus on risk factors for CDI in outbreak situations, it is unknown if these risk factors are similar in a setting where CDI only sporadically occurs.

Chapter 5 focuses on the most important risk factor for nosocomial CDI: antibiotic therapy. Virtually all antibiotic classes increase a patient's risk for CDI and a longer duration and higher dose of antibiotic therapy are associated with a higher risk. In this chapter we confirm the aforementioned statements and we determine the duration of the period of increased risk for CDI after antibiotic therapy.

Chapter 6 addresses clinical characteristics of patients with CDI in the community and highlights how these characteristics differ from other patients with diarrhoea who visit a general practitioner. Additionally, current testing algorithms are evaluated and an advice is formulated to how CDI patients in the community can be recognized.

Part II: Course and outcome of *Clostridium difficile* infections

Chapter 7 determines how many CDI patients die in the first 30 days (and one year) after CDI diagnosis. By comparing CDI patients to control patients, we estimate the CDI-related mortality.

Chapter 8 explores if three recently proposed markers for 'severe' CDI can be applied to predict which patients develop a complicated course of CDI. Fever, renal failure and leukocytosis were explored in a large database of two recently completed trials and the database of an observational study from the UK.

Chapter 9 explores if the presence of binary toxin in *C. difficile* strains is associated with a higher mortality within 30 days after diagnosis. In our analysis, we distinguish *C. difficile* isolates of PCR ribotype 027 from other binary toxin positive isolates.

Chapter 10 develops a prediction rule that identifies patients with a high risk to develop a complicated course of CDI. We aimed to construct this rule by using only variables that were available at the bedside of a patient.

Discussion

Chapter 11 summarizes the conclusions of this thesis and critically assesses its conclusions and methodology. Besides, it gives recommendations for future research.

Chapter 12 provides an overview of the content of this thesis in Dutch.

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Chapter 2

Decrease of hypervirulent *Clostridium difficile*

PCR ribotype 027 in the Netherlands

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Eurosurveill 2009

Abstract

After the first outbreaks of *Clostridium difficile* PCR ribotype 027 (North American pulsed-field type 1, restriction endonuclease analysis group BI) in the Netherlands in 2005, a national surveillance programme for *C. difficile* infection (CDI) was started. Furthermore, national guidelines were developed to rapidly recognise type 027 infections and prevent further spread. The mean incidence of CDI, measured in 14 hospitals, remained stable throughout the years: an incidence of 18 per 10,000 admissions was seen in 2007 and 2008. Between April 2005 and June 2009 a total of 2,788 samples were available for PCR ribotyping. A decrease was seen in the number and incidence of type 027 after the second half of 2006. In the first half of 2009, the percentage of type 027 isolates among all CDI decreased to 3.0%, whereas type 001 increased to 27.5%. Type 014 was present in 9.3% of the isolates and *C. difficile* type 078 slightly increased to 9.1%. We conclude that currently there is a significant decrease in type 027-associated CDI in the Netherlands.

Since the new hypervirulent strain of *Clostridium difficile*, PCR ribotype 027, North American pulsed-field type 1 (NAP1), restriction endonuclease analysis (REA) group BI, was found in the United States and Canada in 2001, a large number of countries worldwide reported *C. difficile* infections (CDI) due to this type[1, 2]. Several reports indicated that CDI due to type 027 is associated with a higher morbidity and mortality and also has the tendency to relapse more frequently[3-6]. An overview published in July 2008 revealed that type 027 was detected in 16 European countries and was associated with outbreaks in Belgium, Finland, France, Germany, Ireland, Luxembourg, the Netherlands, Switzerland and the United Kingdom[7]. As of July 2008, outbreaks have also been reported in Austria[8] and Denmark[9].

Soon after the first outbreaks in the Netherlands in 2005, a national surveillance programme for *C. difficile* was initiated by the Leiden University Medical Centre (LUMC) and the Centre for Infectious Disease Control of the National Institute for Public Health and the Environment. All medical microbiologists in the Netherlands were requested to send *C. difficile* isolates to the Dutch national reference laboratory at the LUMC for rapid PCR ribotyping and characterisation in case of an outbreak (more than two CDI cases within one week in one department) or when a patient suffered from severe CDI. In addition, a prospective, three year-long surveillance study of the incidence of CDI and the distribution of the *C. difficile* PCR ribotypes was started in 14 Dutch hospitals in June 2006.

In the period between April 2005 and June 2009, a total of 3,137 samples were submitted to the reference laboratory, of which 89% (n=2,788) were available for PCR ribotyping. Of those 2,788 samples, 51% had been submitted by medical microbiologists because of either severe disease or a CDI outbreak, whereas the remaining 49% were part of the national surveillance study. Since no difference in the distribution of various PCR ribotypes was found between the two surveillance systems, we represent the data combined. The reason for this equal distribution is that most hospitals that encountered an outbreak or a case of severe CDI, continued to submit samples on a regular basis thereafter.

Figure 1 depicts the distribution of the five most common PCR ribotypes in the Netherlands between April 2005 and June 2009. Although the total number of submitted samples increased from 35 in the second quarter of 2005 to a steady number between 150 and 250 after the first quarter of 2006, a decrease in the number of type 027 isolates has been observed since the second half of 2006. In the 14 hospitals participating in the continuous surveillance, a decrease in the quarterly incidence of type 027 (number of isolates per number of admissions) was seen.

This decrease was confirmed in linear regression and remained significant after adjustment for the number of samples that we received (p=0.03).

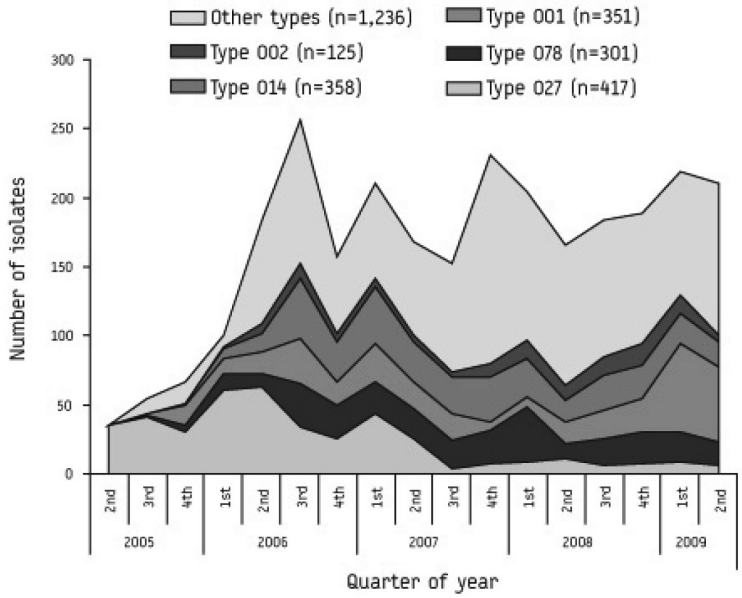

Figure 1. *C. difficile* PCR ribotypes in the Netherlands, April 2005 – June 2009 (n=2,788)

In the first half of 2009, type 027 was found in 3.3% of the 430 submitted samples. Type 001 (n=118; 27.4%) was the most common type, followed by type 014 (n=40; 9.3%), 078 (n=39; 9.1%) and 002 (n=19; 4.4%). We also encountered a number of isolates that did not match a PCR ribotype in our database and belonged to different, yet unknown types (n=49; 11.4%). These are currently subject of further investigation. Finally, of all isolates in the first two quarters of 2009, 35.1% belonged to 41 different PCR ribotypes, which were present in small numbers. Types 015 (n=15; 3.5%), 056 and 087 (both 2.6%), 017 and 046 (both 1.9%) were the five most frequently found types among those. The types that could not be matched in our database and the 41 less common types were combined in the group 'other types', as displayed in figure 1.

To determine the incidence of CDI in the Netherlands, we used the continuous surveillance data only. From the beginning of 2007 to the end of 2008, the mean incidence was 18 per 10,000 hospital admissions, ranging from 8 to 35 per 10,000 admissions among the 14 hospitals. These numbers are in line with a previous study performed in the Netherlands, which showed an incidence of 16

per 10,000 admissions[10]. A nationwide incidence study in neighbouring Belgium revealed a similar (median) incidence of 15 per 10,000 admissions[11].

Discussion and conclusions

To our knowledge, the Netherlands are the first European country with a documented decrease of the hypervirulent type 027. The detection of type 027 in 2005 resulted in a number of measurements taken on a national level. Most hospitals which experienced CDI due to type 027 followed the principles of the infection control guideline supported by the European Centre for Disease Prevention and Control (ECDC) to limit the spread of *C. difficile*, emphasising the importance of responsible use of antimicrobial drugs in conjunction with proper environmental disinfection, compliance with hand hygiene, protective clothing, education of staff and single-room isolation or cohorting of CDI patients[12, 13]. Although the role of fluoroquinolones as an important predisposing factor for CDI due to type 027 has been recognised in several outbreaks[13, 14], the observed decrease in incidence of type 027 in the Netherlands is not related to a change of nationwide use of fluoroquinolones since this remained stable in hospitals[15].

The relatively high frequency of type 001 in Dutch hospitals is not exceptional and has recently also been reported in southern Germany, Ireland, Luxembourg and the United Kingdom[7, 16]. Type 014 is also frequently found in other European countries: it is the most common strain found in Hungary (2002-2004), Norway and Sweden (2008), and the second most common strain in Austria (2006) and Poland (2002-2003)[7, 8, 17, 18]. An increase of type 078 had been noticed previously in the Netherlands[19]. In the quarterly data presented here, the increase is also seen: in the first trimester of 2008 19% of all samples consisted of type 078. After this peak, however, the contribution of type 078 decreased and it became the third most common strain in the Netherlands. Also in several other European countries type 078 is increasingly observed[7]. This type is a predominant strain in some farm animals (especially in pigs and dairy calves) and has recently been found in retail meat in North America[20]. The genetic similarity between animal and human type 078 strains as demonstrated by the highly discriminatory multilocus variable number of tandem repeats analysis (MLVA), also suggests a possible common source of animal and human type 078 strains. Type 078 and type 027 have similar virulence factors (positive for toxin A, B and binary toxin, and a dysfunctional toxin regulator gene). Furthermore, they resemble CDI in their clinical presentation: both cause severe

diarrhoea in 40% of cases. A complicated course is seen less often in CDI caused by type 078, possibly because type 078 is observed in a younger population, with a higher frequency of community-associated CDI[19].

In conclusion, CDI caused by the hypervirulent 027 strain is now observed less frequently in the Netherlands, while the 'common' types 001 and 014 remain prominently present in the Dutch hospitals. Type 078 is currently the third most common PCR ribotype in the Netherlands and other European countries, whereas its occurrence before 2005 was very rare. More research is needed on the source of this strain and a possible exchange between animals and humans.

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Chapter 3

Clostridium difficile infection in the community:

a zoonotic disease?

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Clin Microbiol Infect 2012

Abstract

Clostridium difficile infections (CDI) are traditionally seen in elderly and hospitalized patients who have used antibiotic therapy. In the community, CDIs requiring a visit to a general practitioner are increasingly occurring among young and relatively healthy individuals without known predisposing factors. *C. difficile* is also found as a commensal or pathogen in the intestinal tracts of most mammals, and various birds and reptiles. In the environment, including soil and water, *C. difficile* may be ubiquitous; however, this is based on limited evidence. Food products such as (processed) meat, fish and vegetables can also contain *C. difficile*, but studies conducted in Europe report lower prevalence rates than North America. Absolute counts of toxigenic *C. difficile* in the environment and food are low, however the exact infectious dose is unknown. To date, direct transmission of *C. difficile* from animals, food or the environment to humans has not been proven, although similar PCR ribotypes are found. We therefore believe that the overall epidemiology of human CDI is not driven by amplification in animals or other sources. As no outbreaks of CDI have been reported among humans in the community, host factors that increase vulnerability for CDI might be of more importance than increased exposure to *C. difficile*. Conversely, emerging *C. difficile* type 078 is found in high numbers in piglets, calves and their immediate environment. Although no direct evidence proving transmission to humans, circumstantial evidence points towards a zoonotic potential of this type. In future emerging PCR ribotypes, zoonotic potential needs to be considered.

Introduction

Clostridium difficile is an anaerobic, spore-forming bacterium that can produce toxin A or B upon colonization of the gut. Patients at risk for *C. difficile* infection (CDI) subsequently develop diarrhoea or, in severe cases, a pseudomembranous colitis. Traditionally, elderly and hospitalized patients who had used antibiotic therapy were considered to be the most vulnerable to CDI[3]. Because these high risk patients are primarily located in healthcare facilities, CDI was regarded as a primarily nosocomial disease for many years. This concept is now being challenged, because persons outside hospitals are increasingly developing CDI[4-7].

When CDI is acquired in a healthcare facility, symptoms may start during hospitalization, but they may also develop after discharge. Subsequently, 25 to 50% of the patients who develop CDI outside a hospital have had a recent hospital admission[5, 8-10]. A clear definition of CDI is necessary to distinguish between healthcare-acquired CDI and community-acquired CDI (CA-CDI). For this review, we define CA-CDI as follows: patients with symptoms of CDI starting in the community or within 48 hours of admission to a healthcare facility, provided that the onset was more than 12 weeks after the last discharge from a healthcare facility, according to guidelines from the European Centre for Disease Prevention and Control and the CDC[1, 2]. Some studies included in this review have modified this definition (Tabel 1).

Besides its presence in humans, *C. difficile* has also been described as a commensal or pathogen in numerous animal species. Because patients with CA-CDI do not, by definition, acquire *C. difficile* in a hospital, the question arises as to what the source of exposure might be in the community. Direct or indirect contact with animals was proposed as a possible source of *C. difficile*. This review describes the occurrence of CA-CDI and discusses the potential sources of *C. difficile* in the community. Furthermore, it summarizes the evidence for *C. difficile* being considered as a new zoonotic agent.

Table 1. Incidence of Clostridium difficile infections in the community and the association with antibiotic usage.

			CDI patients						
Author	**Study period**	**Setting**	**(N)**	**Incidence**	**Population tested**	**Definition of CA-CDI**	**Test for C difficile**	**Prior antibiotic usage**	**Median age CDI patients (y)**
Riley et al.[25]	1983-1984	GP/small hospital, Australia	89 (36 toxi-genic)	4.7% (2.1% toxigenic)	Diarrhoeal samples submitted by GP/hospital	CO-CDI	Culture, followed by cytotoxicity assay	-	-
Riley et al.[26]	1988	GP, Australia	16	5.6%	Diarrhoeal samples submitted by GP	CO-CDI	Selective enrichment broth and latex agglutination test	69% (3 months)	-
Hirschhorn et al.[17]	1988-1990	GP/ hospital, USA	51	7.7 / 100,000	Upon request	CO-CDI or onset within 48 hours of admission, no hospitalisation previous 6 weeks, or diagnosis and symptoms within 5 days of admission	Cytotoxicity assay	65% (42 days)	37
Khanna et al.[7]	1991-2005	GP/ hospital, USA	157	2.8 / 100,000 in '91-'93 15 / 100,000 in '03-'05	Upon request	CO-CDI, or onset within 48 hours of admission, no hospitalisation previous 4 weeks	Cytotoxicity assay, switched to enzyme immunoassay	78% (3 months)	50 (mean)
Barrett et al.[18]	1994	GP, UK	7	5.8%, 13.6% (2 districts)	Upon request	CO-CDI	-	-	-
Riley et al.[24]	< 1994	GP, Australia	13 62	2.6% 10.7%	Feacal samples submitted by GP Upon request or after antibiotic use	CO-CDI, no recent hospitalisation CO-CDI, no recent hospitalisation	Vero cell assay and culture	85% (4 weeks)	43 (mean)
Wheeler et al.[22]	1993-1997 1993-1997	commu-nity, UK GP, UK	6 17	160 / 100,000 20 / 100,000	Diarrhoeal patients Diarrhoeal patients	CO-CDI CO-CDI	Vero cell assay Vero cell assay	- -	- -
Karlstrom et al.[13]	1995	GP/ hospital, Sweden	529	20 / 100,000	Upon request	CO-CDI, no hospitalisation previous 4 weeks	Enzyme immunoassay, sometimes with culture	88% (6 weeks)	59
Dial et al.[5]	1994-2004	GP, UK	1233	<1 / 100.000 in 1994 18 / 100.000 in 2004	Upon request	CO-CDI no hospitalisation previous year	Non-specified toxin test	36% (3 months)	71 (mean)

Author	Study period	Setting	(N)	CDI patients Incidence	Population tested	Definition of CA-CDI	Test for C difficile	Prior antibiotic usage	Median age CDI patients (y)
Wilcox et al.[23]	1999	GP, UK	42	24 / 100,000	Diarrhoeal samples submitted by GP	CO-CDI	Vero cell assay and culture	52% (1 month)	<65
Noren et al.[12]	1999-2000	GP/ hospital, Sweden	59	25 / 100,000	Upon request	CO-CDI, no hospitalisation during study period	McCoy cell assay and culture	-	64
Forward et al.[21]	1999-2000	GP, UK	5	0.6%	Diarrhoeal samples submitted by GP	CO-CDI	Vero cell assay	100% (1 month)	>60
Hirshon et al.[27]	2002-2007	primary care clinic/ hospital, USA	43	3.9%	Diarrhoeal outpatients	CO-CDI	Enzyme immunoassay	63% (1 month)	44 (mean)
Kuntz et al.[19]	2004-2007	GP/ hospital, USA	304	11 / 100,000	Upon request	CO-CDI, no hospitalisation in preceding 12 weeks	-	73% (6 months)	between 19-49
Lambert et al.[14]	2005-2006	GP/ hospital, Canada	275	23 / 100,000	Upon request	CO-CDI, or onset within 48 hours of admission, no hospitalisation previous 12 weeks	Enzyme immunoassay	-	<60
Anonymous (MMWR)[20]	2006	GP/ hospital, USA	241	6.9 / 100,000	Upon request	CO-CDI, or onset within 48 hours of admission, no hospitalisation previous 12 weeks	Non-specified toxin test	68% (3 months)	between 45-64
Huhulescu et al.[29]	2007	GP, Austria	14	236 / 100,000 4.6%	Diarrhoeal patients	CO-CDI	Culture followed by immunocard	38% (2 months)	36
Bauer et al.[32]	2008	GP, the Nether-lands	37	1.5%	Diarrhoeal samples submitted by GP	CO-CDI	Enzyme immunoassay	58% (6 months)	54
Fellmeth et al.[15]	2008-2009	GP, UK	54	1.5% 13 / 100,000	Diarrhoeal samples submitted by GP of patients <65 years	CO-CDI	Enzyme immunoassay	32% (4 weeks)	between 31-40

CA-CDI, community-acquired CDI; CO-CDI, CDI that started in the community. Studies that used this definition only, disregarded the presence of a recent hospital admission. GP: general practice.

Occurrence of CDI in the community

CDI is frequently diagnosed within healthcare facilities, and the incidence can rise above 200 per 10,000 admissions[11]. The incidence of CDI occurring outside healthcare facilities is significantly lower[12]. Nevertheless, CDI acquired in the community accounts for one-quarter of all diagnosed CDI patients[7, 12, 13]. Table 1 summarizes studies investigating the incidence of CA-CDI, and shows the study population, the definition of CA-CDI applied, the test that was used, and the incentive to test patients for CDI. In four studies, an enzyme immunoassay was used to diagnose CDI. Enzyme immunoassays have been criticized recently for their low sensitivity, despite their good specificity[10, 13-15]. However, even a relatively specific test will give false positive results in a low-incidence setting such as the community[16], and this will impact on a reliable estimation of the incidence. The incentive to test for CDI also affects the incidence. When patients are tested only upon request of the physician, patients without known risk factors for CDI may be missed. Half of the studies reported in Table 1[5, 7, 12-14, 17-21] only tested faeces samples for *C. difficile* on request of the physician. Despite the relatively similar incidence rates that are reported, most studies are likely to under-report the real occurrence of CDI, owing to their methodological weaknesses.

The population-based study by Wheeler et al.[22] was the only study that tested all diarrhoeal patients in the community, regardless of whether patients visited a general practitioner (GP) or whether CDI was suspected. Between 1993 and 1997, they included 9776 patients, randomly selected from the GPs' patient lists, and prospectively questioned them about the occurrence of diarrhoea during six consecutive months. Diarrhoea occurred in 781 cases and six of these patients were found to be positive for *C. difficile* by the use of Vero cells, resulting in an incidence of 160 per 100,000 persons per year. Microbiological studies in the community are scarce, and all other studies in Table 1 were performed among patients with diarrhoea visiting their GP. These studies report an incidence of CDI of 7 to 25 per 100,000 persons per year[5, 12-14, 17, 19, 20, 22, 22, 23], which is eight-fold lower than the incidence found in the community. This difference suggests that many patients do not seek medical attention for mild diarrhoea caused by *C. difficile*[22]. When the number of people serviced by a laboratory or hospital (catchment area) is unknown, incidences cannot be determined, and only the percentage of positive tests can be reported. Patients presenting to the GP with diarrhoea have a positive test result for CDI in 2 to 6% of the cases[10, 18, 24-27]. This increases to approximately 10% when

antibiotics are used or a physician specifically requests testing, often because risk factors are present[24, 28].

Campylobacter, *Salmonella*, *Yersinia* and *Shigella* were more frequent causes of diarrhoea diagnosed by GPs, according to Wheeler et al.[22] An Australian study detected *C. difficile* in 89 patients, and 36 strains produced toxins (2.1% of total). Toxigenic *C. difficile* was also less frequent than *Campylobacter* (3.2%), *Shigella* (3.2%) and *Salmonella* (2.9%) in this study.[25] Both studies were performed before the incidence of CDI increased worldwide in the beginning of the 20th century. In 2007, a small Austrian study concluded that CDI was the most frequent bacterial cause of gastroenteritis in general practice.[29] However, this finding should be interpreted with caution as the incidence of CDI was extraordinary high (236/100,000), possibly due to the use of a test with a low positive predictive value[30] (ImmunoCard, Meridian Bioscience, Cincinnati, USA) and the inclusion of patients with a history of recent hospital admission[29].

Studies on patients with severe community-acquired diarrhoea requiring hospital admission who were subsequently diagnosed with CDI are not given in Table 1. It is estimated that these patients account for over 5% of all hospitalized CDI patients, emphasizing the importance of better guidelines for the diagnosis CA-CDI[14, 31]. Kuntz et al.[19] and Riley et al.[24] reported that 6% of the CA-CDI patients are treated for a recurrence. Bauer et al.[10] found a higher recurrence rate (29%); however, this study also included patients with a recent hospital admission. Although about 10% of the CA-CDI patients who are diagnosed by their GPs are hospitalized during the course of their disease[17, 23], CDI-related mortality rates in this group of patients are very low (≤3%)[23, 31, 32].

Patient and strain characteristics in CA-CDI

Patients with CA-CDI do not have the classic risk profile of patients who develop CDI in a healthcare facility. Only 32 to 88% used antibiotic therapy before their diarrhoea, and the mean age was below 65 years in all but one study (Table 1)[5]. Four studies that reported relatively low antibiotic usage might have suffered from patient recall bias[15, 27, 29, 32]. However, a large study by Dial et al. that used a drug prescription database also concluded that only 36% of the patients with CA-CDI used an antibiotic. Therefore, the variations in reported antibiotic use are probably attributable to the varying time intervals in which data were gathered and the differences in study populations. In a statewide surveillance study in Connecticut, 241 cases with confirmed CA-CDI

were reported by healthcare providers to the Department of Public Health. One-quarter of them had no underlying illness or hospitalization in the preceding year.[20] Similar results were seen in four other studies, where 16%, 26%, 35% and 40% had no antibiotic use or admission preceding their CA-CDI[23, 27, 32, 33]. Furthermore, severe CDI was reported among previously low risk populations, such as healthy individuals and pregnant women[4]. The emergence of CDI among pregnant women was only reported in two small studies of ten patients[4, 34], the majority of whom had a history of prior antibiotic therapy. Larger studies have not confirmed the emergence of CDI in pregnancy. The susceptibility to CDI of patients without traditional risk factors is not well understood. Proton pump inhibitors, which are used to treat reflux disease and peptic ulcers, were postulated to increase the vulnerability to *C. difficile*. Several studies reported discrepant results, and there is no consensus on whether this frequently used treatment predisposes to CDI[5, 31, 35, 36]. Identification of additional factors that increase vulnerability is therefore needed. The selection of an appropriate control group is essential for this purpose. Many recent studies compared hospitalized CDI patients with CDI patients from the community, which will not result in identification of new risk factors.

In order to explain the emergence of CA-CDI, new routes of transmission have been considered. A disease transmission model proposed by Otten et al.[37] mentioned four potential sources: the environment, contact with infected or colonized patients, contact with infected or colonized animals, and foodborne transmission. Increased exposure to one or more of these sources might explain the increase in the number of cases of diagnosed CA-CDI. However, as no outbreaks of CDI have been reported in the community, host factors that increase vulnerability might be of more importance in development of CDI than increased exposure to *C. difficile*.

A study of 57 patients with CA-CDI who were diagnosed by their GPs showed an association between CDI and contact with infants under 2 years of age in univariate analysis[23]. This association had not been found previously, possibly because it had not been looked for. The absence of a multivariate analysis implies that this association could have resulted from confounding. However, infants are known to be frequently colonized (approximately 40%) with toxigenic *C. difficile*[38]. These children rarely develop symptoms, and this is hypothesized to be attributable to the lack of a receptor for toxin A, but evidence for this hypothesis is lacking.

Information on the strains of *C. difficile* isolated from patients with CA-CDI is scarce and available from only a few small studies. The most frequently found PCR ribotypes were 078, 001 and 014[10, 39, 40]. These ribotypes are also among the most

prevalent in hospitals[41, 42]. Ribotype 027, however, was also found in smaller numbers than in hospitals.[39, 43] Strains such as ribotype 027, especially its spores, spread more easily within the hospital, because they can resist the hospital environment, cleaning, and disinfectants[44]. Variation in antibiotic prescriptions might account for the higher prevalence of type 027 in hospitals.

Clostridium difficile in animals and potential for transmission

CDI in animals was unknowingly described in 1968 when Small et al.[45] reported a case of fatal enteritis in laboratory hamsters after administration of antibiotics. Since then, hamsters have been used as animal models to prove the association of *C. difficile* with pseudomembraneous colitis in humans.[46] *C. difficile* has been isolated from almost all mammals[47, 48], including cows, horses[49], pigs[50], elephants[51], Kodiak bears[52] and non human primates[53], and also poultry[54] and ostriches[55]. In contrast to human medical research, where studies are mostly focused on the role of *C. difficile* in disease, many studies in animals concentrate on the presence of the bacterium in healthy animals. Investigations on the role of household pets as a possible reservoir of *C. difficile* showed that both healthy and diseased dogs and cats can shed spores of *C. difficile*[56, 57]. Riley et al.[57] sampled dogs and cats that were treated for a variety of reasons at two veterinary clinics, using selective solid and enrichment media, and found *C. difficile* in 39.5%. At both clinics, the environment became grossly contaminated with *C. difficile* as 40% and 75% of the sites were positive. Both toxigenic and non-toxigenic *C. difficile* isolates were recovered, but no overlap between animal and human isolates of *C. difficile* was found after typing[58]. In 2010, *C. difficile* colonization of pets and contamination of households was again evaluated by Weese et al.[59]. In 26 (31%) of the 84 households that were sampled, 14 (10%) of 139 dogs and three (21%) of 14 cats were positive for *C. difficile*. Again no overlap between canine strains and environmental isolates was seen after PCR ribotyping. In contrast to other studies, where the predominant ribotype in dogs and cats was the non-toxigenic 010, the most common ribotype in dogs and cats in this study was 001[60, 61]. This was also the most common ribotype among humans in the study area[59]. In fact, all toxigenic strains isolated from the pets in this study are known to be implicated in human CDI.

PCR ribotypes known to be involved in human CDI were also isolated from horses[60, 62]. Keel et al.[60] and Koene et al.[61] reported a diversity of ribotypes (>10 different types) in horses. Ribotype 015 was predominant according to Keel et al.,

whereas the Dutch study did not find a predominant ribotype. Songer et al.[63] reported a case of fatal typhlocolitis caused by ribotype 027 in a 14-year-old quarter horse. *C. difficile* seems to be a rare finding in healthy adult horses, as a low prevalence (0-1.2%) is reported for horses without signs of diarrhoea[64, 65]. The prevalence of the bacterium is higher in adult horses with diarrhoea and in foals, where it varies from 6% to 40%[64, 65].

Most of the published research on *C. difficile* in animals has been focused on production animals. The first large-scale study in food-producing animals was conducted in 1996 by Al Saif and Brazier[50], and although at least 100 animals from every animal species in the study, i.e. cattle, sheep, poultry, pigs and horses, from 40 different farms were sampled, *C. difficile* was isolated only rarely. The highest prevalence was found in poultry (1.6%) and the bacterium was not isolated from the pigs or cattle[50]. The age of the sampled animals was unknown, and because older age in animals is associated with a low *C. difficile* prevalence[66], the results of this study could be due to an age effect. Since the beginning of the 21st century the epidemiology of *C. difficile* in production animals has changed, because *C. difficile* is increasingly reported as a major cause of neonatal enteritis in piglets[67-69]. Even though the postulate of Koch was confirmed in two different studies in which piglets inoculated with *C. difficile* spores developed characteristic gross and microscopic signs of disease[68, 70], the role of the bacterium in disease in pigs is still questioned, since no association between diarrhoea and presence of the bacterium was found in a large Spanish study[71]. No clear correlation between disease and the presence of the bacterium was found in calves either[72, 73]. The ribotypes of isolates originating from cattle and pigs are much less diverse than those in dogs, horses, and humans.[60] The predominant PCR ribotype is 078, which accounted for 94% and 83% of the bovine and swine isolates in the study by Keel et al.[60] and for 100% of the isolates in a study by Keessen et al.[74]. In poultry, the association between enteritis and colonization with *C. difficile* is less well studied[54, 75, 76]. Zidaric et al. ribotyped 44 isolates from two separate flocks at one poultry farm. A wide variety of 12 different ribotypes was found, with none of them being predominant[54]. An overview of the predominant ribotypes of *C. difficile* in animal species is given in table 2.

Table 2. *Clostridium difficile* in animal species.

Animal species	Predominant Ribotype	Frequency N type/ total (%)	Study period	Reference
dogs	010	5/12 (42)	2007 (published)	Keel et al.[60]
	010	12/29 (41)	2009-2010	Koene et al.[61]
	001	4/14 (29)	2005-2006	Weese et al.[59]
	014	7/29(24)	2009-2010	Koene et al.[61]
cats	010	9/18 (50)	2009-2010	Koene et al.[61]
	039	5/18((28)	2009-2010	Koene et al.[61]
horses	015	6/20 (30)	2007 (published)	Keel et al.[60]
pigs	078	33/33 (100)	2008 (published)	Debast et al.[80]
	078	66/66 (100)	2009	Keessen et al.[74]
	078	93/144 (84)	2007 (published)	Keel et al.[60]
	078	7/9 (78)	2009-2010	Koene et al.[61]
	066	166/247 (67)	2009 (published)	Avbersek et al.[62]
	066	66/133 (50)	2008 (published)	Pirs et al.[111]
	SL011*	74/247 (30)	2009 (published)	Avbersek et al.[62]
	SL011*	31/133 (23)	2008 (published)	Pirs et al.[111]
	126	16/144 (11)	2007 (published)	Keel et al.[60]
	002	6/144 (4)	2007 (published)	Keel et al.[60]
	029	7/247 (3)	2009 (published)	Avbersek et al.[62]
cattle	078	31/33 (94)	2007 (published)	Keel et al.[60]
	078	31/33 (94)	2008 (published)	Hammitt et al.[112]
	012	5/6 (83)	2009-2010	Koene et al.[61]
	017	8/31 (26)	2004	Rodriguez-Palacios et al.[72]
	078	7/31 (23)	2004	Rodriguez-Palacios et al.[72]
	027	4/31 (13)	2004	Rodriguez-Palacios et al.[72]
	014	4/31 (13)	2004	Rodriguez-Palacios et al.[72]

* this type could not be identified.
Only when a ribotype was encountered in at least 4 animals per animal species, results were included in this table.

Although the issue of zoonotic transmission of *C. difficile* was raised more than 20 years ago, and the finding of overlapping ribotypes in animals and humans has stimulated research in this field, the question of whether zoonotic transmission occurs has not been answered. Circumstantial evidence that *C. difficile* strains from animals were infecting humans (or vice versa) has been reported several times in recent years[60, 77]. These studies have taken animal and human isolates and typed them by molecular methods, and have shown overlap between isolates in the two groups. For example, Arroyo et al.[77] looked at 133 isolates of *C. difficile* from dogs (n=92), horses (n=21) and humans (n=20), plus one each from a cat and a calf. Overall, 23 different ribotypes were identified. Of these, nine were identified from dogs, 12 from horses, seven from humans, and one each from the cat and calf. Although absolute numbers were small, 25% of the human isolates were indistinguishable

from animals isolates according to PCR ribotyping. Keel et al.[60] examined a similar number of isolates (n=144) and again showed similarities between horse, dog and human strains of *C. difficile* with PCR ribotyping, but not with strains from cattle or pigs. Other, more discriminatory, typing methods for *C. difficile,* such as multilocus variable-number tandem-repeat analysis or microarrays, also showed overlap between human and animal isolates[78-80]. Whether *C. difficile* strains in humans and animals are really identical should be determined by, for example, whole genome sequencing. The similarities seen in strains of human patients and different animal species do not automatically imply that interspecies transmission occurs. However, as living with an immunocompromised person is a risk factor for colonization with *C. difficile* for dogs[59], and the risk of *C. difficile* colonization of hospital visitation dogs is associated with close human contact[81], interspecies transmission is likely to occur.

In The Netherlands an overlap between the location of pig farms and the occurrence of human *C. difficile* ribotype 078 infections, which are increasing in prevalence, is observed[82]. The fact that infections with ribotype 078 in humans occurred in a younger population and were more frequently community-acquired than infections with ribotype 027 strains, together with the fact that 078 is the predominant ribotype in piglets, suggested a common source[82]. This common source is likely to be the environment. If infection rates in pig farms in the Netherlands are as high as those in the USA[67], it is likely that a large proportion of the Dutch population comes into contact with *C. difficile* spores every day, especially since the Netherlands has one of the highest population densities in the world. There is little evidence that other epidemic strains have zoonotic potential.

Environmental contamination

Because of its spore forming ability, *C. difficile* can survive in the environment for several months. The presence of *C. difficile* spores in hospitals is well established[83]. Also, gross contamination of farms such as pig facilities with *C. difficile* spores is commonplace. *C. difficile* could be isolated from the faeces of piglets 1 h after birth, presumably ingested from their environment. Within 2 days of birth, 100% of piglets had acquired *C. difficile* of the same molecular type that was found in sow faeces, sow teats, farrowing crates, and air on the farm[84]. There is evidence that vertical transmission does not occur in pigs[84]. Aerial dissemination of *C. difficile* on a pig farm has been shown to correlate with the activity of personnel within farrowing units[85], suggesting that staff might be at increased risk of ingesting airborne *C. difficile*

spores. Contamination of the pig farm environment was confirmed in another study where *C. difficile* prevalence in the environment increased from 0% to 61% of sites within a pig farrowing facility only 1 month after it has been occupied with pigs[86]. *C. difficile* spores and vegetative cells are shed into the immediate environment in the faeces of both scouring and non-scouring pigs, underscoring the importance of high carriage rates in apparently healthy piglets[84]. The carrier state is also emphasized in mouse studies that have demonstrated a marked increase in spore shedding when antibiotics are given to asymptomatic carrier mice. Subsequent spore-mediated transmission to immunosuppressed mice led to severe intestinal disease[87]. Another important consideration in relation to environmental contamination is effluent arising from piggeries. In Australia, piggery effluent is treated in anaerobic ponds to remove pathogens, and re-used to wash sheds or applied to agricultural land. *C. difficile* was shown to survive this process, with concentrations of viable *C. difficile* spores of greater than 200 CFU/mL (Squire and Riley, unpublished) posing a risk for infection of animals or contamination of agricultural produce.

Besides environmental contamination in the vicinity of colonized or infected humans and animals, *C. difficile* spores can be isolated from practically any environmental site, provided that the correct culture enrichment methods are employed[88]. A large study by Al Saif and Brazier[50] showed high rates of detection of *C. difficile* in soil and water samples in South Wales. Soil contained *C. difficile* in 21% of 104 samples, and 41% of the isolates produced toxin A. Water was positive in 88% of river samples, half of the sea, lake, and swimming pool samples, and 5.5% of the tap water samples. Overall, 85% of the isolates produced toxin A. In 2010, similar percentages were found in Slovenia[89], where 61% (42 of 69) of the river isolates was positive for *C. difficile*. Interestingly, 34 different types were found, more than half of which were also found in humans and animals. Ribotype 014, a common ribotype found in humans, was the most prevalent (16%). Although absolute counts of toxigenic *C. difficile* in water are low (1-5 CFU/100ml)[50], the infectious dose is unknown, and therefore so is the impact of the environment as a source of human or animal CDI.

C. difficile in food products

As *C. difficile* can be detected in live animals, foodborne transmission via meat is also considered to be a potential source of CA-CDI. Recently, a number of studies

have been published on the prevalence of *C. difficile* in (processed) meat, fish, and vegetables. These results are summarized in Table 3.

Remarkably, studies conducted in Europe persistently reported low prevalence rates, e.g. in up to 3% of meat samples[90-95], compared to the USA and Canada where *C. difficile* is generally reported at much higher rates, e.g. in up to 42% of meat samples[96-104]. Although high isolation frequencies are reported for *C. difficile* in meat, quantitative studies show that levels of contamination are generally low, with <100 CFU/g in chicken meat [101] and typically 20 to 240 spores/g in retail beef and pork[99]. Despite the low numbers, the spore forming nature of *C. difficile* and the heat tolerance of the spores[96] might facilitate foodborne transmission[101]. The majority of *C. difficile* isolates that have been recovered from food are toxigenic and therefore potentially pathogenic, with a clear overlap in types being found in human patients. PCR ribotypes 078 and 027 have not been isolated from meat samples in Europe, but are the main ribotypes found in food in North America (Table 3). However, this finding needs to be confirmed, because laboratory cross-contamination may have occurred in some studies[105]. If we exclude the study by Songer et al.[98], who found a high prevalence rate, the overall prevalence rate of *C. difficile* in meat samples in North America drops to 2%-20%, and more resembles the percentages found in Europe. Meat has been given most attention, and limited information is available on other food products. *C difficile* has been found in seafood and fish[50, 106], and also in vegetables[50, 107, 108] and environmental samples[50]. So far, the isolation of *C. difficile* from milk and milk products has not been reported, despite the presence of *C. difficile* in cattle faeces.

Whether the differences observed between countries, both in overall prevalence rates and in ribotypes, truly reflect geographical differences in occurrence, reflect temporal or seasonal differences in prevalent ribotypes or perhaps are caused by other factors is presently unknown and needs further investigation. Conceivably, the differences are affected by the use of different methodologies, although these do not seem to be related to distinct regions. Poor reproducibility with some methodologies has been shown, suggesting that present culture methods might be suboptimal for the detection of *C. difficile* in meat samples[97, 109]. Furthermore, the interpretation of findings is hampered by the use of different sampling methods. Validated methodologies for the sampling and isolation of *C. difficile* from food and environmental samples are urgently needed. The source of contamination with *C. difficile* in retail meats is also presently unknown. It may involve faecal or environmental contamination of carcasses, or contamination during processing by

shedding handlers[96, 98]. In addition, ante mortem deposition of (dormant) spores in the animal's muscle or other tissues has been suggested as a possible food contamination route[97, 109].

Although *C. difficile* is present in food for human consumption, and overlapping PCR ribotypes from animal and human sources have been reported[58, 77, 110], foodborne infection caused by *C. difficile* has never been confirmed. Further studies are required to provide relevant data on the sources, transmission routes, growth and survival of *C. difficile* in foods. Additionally, more information on the infective dose and more quantitative information on the level of contamination are needed to further measure the risks for humans associated with food-borne exposure to *C. difficile*.

Conclusion

C. difficile frequently causes mild, self limiting diarrhoea in the community. Only a minority of these patients seek medical attention. *C. difficile* is also found in animals, food products and the environment. To date, direct transmission from one of these sources to humans has not been proven, and there is little evidence that frequently found PCR ribotypes such as 001, 014 and 027 have a zoonotic source. We therefore believe that the overall epidemiology of human CDI is not driven by amplification in animals. However, because almost all PCR ribotypes are able to colonize or infect different hosts, and host-specific PCR ribotypes do not seem to occur, we assume that zoonotic transmission is possible. The emerging *C. difficile* type 078 in humans is epidemiologically linked to its presence in piglets, calves, and their environment, suggesting zoonotic transmission. Because this evidence is circumstantial, it needs to be determined whether patients at risk for CDI can truly be infected by these animals or their environment. The risk for infection of persons in close contact to these animals is likely to be small, although preliminary data indicate that colonization frequently occurs (Keessen et al, manuscript in preparation). The zoonotic potential of other frequently found pathogenic *C. difficile* ribotypes is probably very low. However, when new PCR ribotypes emerge, zoonotic transmission should always be considered.

Table 3. *Clostridium difficile* in food products.

	Country	Sample material	Npos samples/ Ntested samples (%)	Toxinogenic/ all isolates (%)	RT 078 or related strains* (%)	RT 027 or related strains** (%)	Other toxinogenic types (%)	Reference
Retail beef and veal								
North America	Canada	Ground meat	12/60 (20.0)	11/12 (91.2)	-	8/12 (66.7)	RT 077 (16.7) RT 014 (8.3)	Rodriguez-Palacios et al. (2007)[95]
	Canada	Ground beef and veal chops	13/214 (6.1)	12/15 (80.0)	-	4/15 (26.7)	RT 077 (20.0) RT 014 (13.3) NAP9 (6.7), C (6.7), H (6.7)	Rodriguez-Palacios et al. (2009)[96]
	USA	Cooked and uncooked beef	14/33 (42.4)	14/14 (100)	10/14 (71.4)	4/14 (28.6)	-	Songer et al. (2009)[97]
	Canada	Ground beef	14/115 (12.2)	14/14 (100)	12/14 (85.7)	1/14 (7.1)	Toxinotype IX (7.1)	Weese et al. (2009)[98]
	USA	Ground veal	4/50 (8.0)	3/4 (75.0)	NT	NT	NT	Houser et al. (2011)[103]
Europe	Sweden	Ground beef	2/82 (2.4)	2/2 (100)	NT	NT	NT	Von Abercron et al. (2009)[90]
	France	Ground beef	2/105 (1.9)	2/2 (100)	-	-	RT 012 (100)	Bouttier et al. (2010)[91]
	Austria	Ground beef	0/30 (0)	-	-	-	-	Jöbstl et al. (2010)[93]
		Ground beef/pork	3/70 (4.3)	1/3 (33.3)	-	-	RT 053 (33.3)	
	Netherlands	Beef/calf	0/164 (0)	-	-	-	-	De Boer et al. (2011)[94]
	Switzerland	Ground beef/pork	0/46 (0)	-	-	-	-	Hoffer et al. (2010)[92]
Retail pork								
North America	USA	Cooked and uncooked pork	19/46 (41.3)	19/19 (100)	13/19 (68.4)	6/19 (31.6)	-	Songer et al. (2009)[97]
	Canada	Ground pork	14/115 (12.2)	14/14 (100)	10/14 (71.4)	1/14 (7.1)	Toxinotype IX (7.1) Toxinotype 0 (7.1) Toxinotype III (7.1)	Weese et al. (2009)[98]
	Canada	Ground pork and pork chops	7/393 (1.8)	6/7 (85.7)	-	4/7 (57.1)	Toxinotype 0 (14.3), Toxinotype XXVI (14.3)	Metcalf et al. (2010)[99]
	USA	Pork and swabs	23/243 (9.5)	23/23 (100)	22/23 (95.6)	-	Toxinotype XI (4.3)	Harvey et al. (2011)[101]

	Country	Sample material	Npos samples/ Ntested samples (%)	Toxinogenic/ all isolates (%)	RT 078 or related strains* (%)	RT 027 or related strains** (%)	Other toxinogenic types (%)	Reference
Europe	Austria	Pork	0/27 (0)	-	-	-	-	Indra et al. (2009)[89]
	Austria	Ground beef/pork	3/70 (4.3)	1/3 (33.3)	-	-	RT 053 (33.3)	Jöbstl et al. (2010)[93]
	France	Pork sausage	0/59 (0)	-	-	-	-	Bouttier et al. (2010)[91]
	Switzerland	Ground beef/pork	0/46 (0)	-	-	-	-	Hoffer et al. (2010)[92]
	Netherlands	Beef/calf	0/63 (0)	-	-	-	-	De Boer et al. (2011)[94]
Poultry products								
North America	USA	Turkey	4/9 (44.4)	4/4 (100)	4/4 (100)	-	-	Songer et al. (2009)[97]
	Canada	Chicken meat	26/203 (12.8)	26/26 (100)	26/26 (100)	-	-	Weese et al. (2010)[100]
	USA	Chicken meat	4/32 (12.5)	7/7 (100)	7/7 (100)	-	-	Harvey et al. (2011)[101]
Europe	Austria	Chicken meat	0/6 (0)	-	-	-	-	Indra et al. (2009)[89]
	Netherlands	Chicken meat	7/257 (2.7)	4/7 (57.1)	-	-	RT 001 (14.3), RT 003 (28.6) RT 087 (14.3)	De Boer et al. (2011)[94]
Other food and feed products								
North America	Canada	Vegetables, divers	5/111 (4.5)	2/5 (40)	3/5 (60)	-	NAP4/ Toxinotype 0 (40)	Metcalf et al. (2010)[99]
	Canada	Seafood/fish	5/119 (4.8)	0/5 (0)	4/5 (80)	-	-	Metcalf et al. (2011)[105]
	Canada	Dog and cat feed	1/25 (4)	NT	NT	NT	NT	Weese et al. (2005) 113
Europe	UK	Raw vegetables	7/300 (2.3)	5/7 (71.4)	NT	NT	NT	al Saif and Brazier (1996)[49]
		Fish gut contents	0/107 (0)					
	UK	Ready-to-eat salads	3/40 (7.5)	3/3 (100)	-	-	RT017 (67) RT001 (330)	Bakri et al. (2010)[107]
	Austria	Raw milk	0/50	-	-	-	-	Jöbstl et al. (2010)[93]

* NAP07, Toxinotype V or related strains.
** NAP01, Toxinotype III, M31 or related strains.
NT: not tested.

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Part I

Population at risk for a *Clostridium difficile* infection

Chapter 4

Clostridium difficile infection in an endemic setting in the Netherlands

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Clin Microbiol Infect 2012

Abstract

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

Introduction

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

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

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

Methods

Patients

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

Definitions

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

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

Isolation and characterization of *Clostridium difficile*

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

Data collection

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

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

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

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

Statistical analysis

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

Results

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

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

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

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

Characteristics and risk factors

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

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

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

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

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

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

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

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

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

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

Clinical course

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

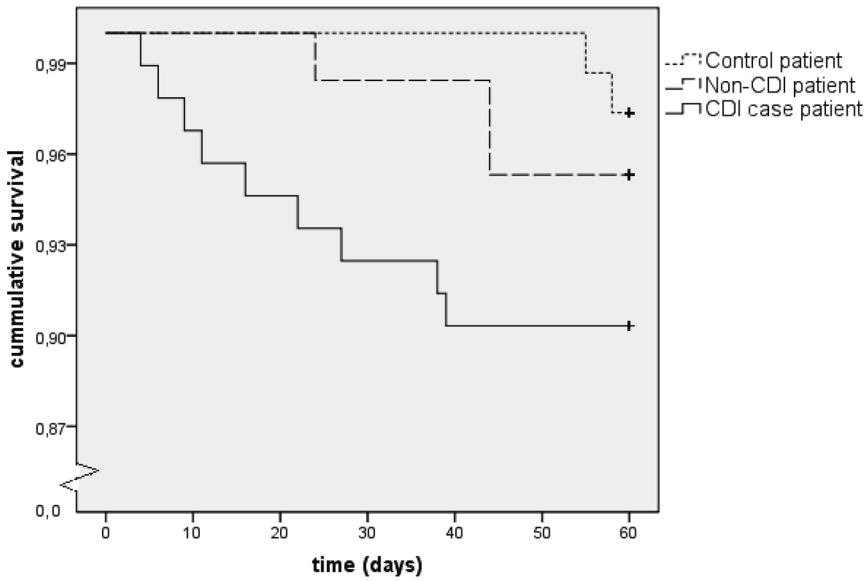

Figure 1. Survival curve of patients with CDI, non-CDI diarrhoea and control patients, in a period of 60 days after the reference date.

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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Chapter 5

Time-interval of increased risk for *Clostridium difficile* infection after exposure to antibiotics

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Clin Microbiol Infect 2012

Abstract

Background: *Clostridium difficile* infections (CDIs) are common in developed countries and affect more than 250,000 hospitalized patients annually in the USA. The most important risk factor for the disease is antibiotic therapy.

Methods: To determine the period at risk for CDI after cessation of antibiotics, we performed a multicenter case-control study in the Netherlands between March 2006 and May 2009. Three hundred and thirty-seven hospitalized patients with diarrhoea and a positive toxin test were compared to 337 patients without diarrhoea. Additionally, a control group of patients with diarrhoea due to a cause other than CDI (n=227) was included.

Results: In the month prior to the date of inclusion, CDI patients more frequently used an antibiotic compared with non-diarrhoeal patients (77% versus 49%). During antibiotic therapy and the first month after cessation of the therapy, patients had a seven to ten-fold increased risk for CDI (OR 6.7-10.4). This risk declined in the period between one and three months after the antibiotic was stopped (OR 2.7). Similar results were observed when the second control group was used. All antibiotic classes, except first generation cephalosporins and macrolides, were associated with CDI. Second and third generation cephalosporins (OR 3.3 and 5.3, respectively) and carbapenems (OR 4.7) were the strongest risk factors for CDI. Patients with CDI used more antibiotic classes and more Defined Daily Doses, compared with non-diarrhoeal patients.

Conclusions: Antibiotic use increases the risk for CDI during therapy and in the period of three months after cessation of antibiotic therapy. The highest risk for CDI was found during and in the first month after antibiotic use. Our study will aid clinicians to identify high risk patients.

Introduction

Clostridium difficile infection (CDI) is an emerging disease in the western world and affects more than 25,000 people annually in England and over 250,000 hospitalized patients per year in the United States.[1, 2] Symptoms vary from mild diarrhoea to a severe pseudomembraneous colitis. Reported mortality due to CDI varies from 6% of the patients in endemic situations to 17% in outbreak settings in which the hypervirulent PCR ribotype 027 (NAP-1) is involved.[3, 4]

Known risk factors for CDI are previous hospitalization, advanced age (>65 years) and, most importantly, the use of antibiotics. Several antibiotic classes have been associated with the development of CDI, including clindamycin, cephalosporins and fluoroquinolones.[5, 6] Furthermore, the number of administered antibiotics, their dosage and the duration of therapy were previously identified as factors determining the risk for CDI.[7-9] An important question that remains unanswered concerns the time-interval of increased risk for CDI after exposure to antibiotics.

In recent studies, patients were defined as 'antibiotic users' when they used an antibiotic 'several days' up to '3 months' before CDI was diagnosed.[10-13] A study among a selected population of elderly patients who were admitted due to severe community-acquired CDI, however, suggested that the period of increased risk for CDI was at least thirty days.[14] Detailed knowledge about the risk of CDI after antibiotic exposure can aid clinicians to select high risk patients, improve antimicrobial stewardship and consequently decrease the incidence of CDI.[15] Furthermore, this knowledge can help future research to operate with a more appropriate definition of antibiotic use. Therefore, we evaluated risk factors for CDI in a multicenter case-control study with special interest for the precise time-interval of increased risk for CDI after exposure to antibiotics. Because diarrhoea (without CDI) is a common side effect of antibiotic use, we additionally evaluated the time-interval of increased risk for diarrhoea in general after exposure to antibiotics.

Methods

Patients and data collection

Between March 1st 2006 and May 1st 2009, a case-control study was conducted in nine Dutch hospitals, including Isala Klinieken (Zwolle), University Medical Center St. Radboud (Nijmegen), Leiden University Medical Center (LUMC; Leiden), VU University Medical Center (Amsterdam), St. Elisabeth Ziekenhuis (Tilburg), Amphia

Ziekenhuis (Breda), Kennemer Gasthuis (Haarlem), Academic Medical Center (Amsterdam) and University Medical Center Utrecht (Utrecht). During a minimum of six consecutive months (within the study period of more than three years), a participating hospital included all hospitalized CDI patients in the study. According to the proposed definitions, case patients were defined as patients with diarrhoea and a positive test for *C. difficile* toxin.[16] Diarrhoea was defined as three or more unformed stools (taking the shape of the container) per day. For each CDI patient, two control patients were selected: one patient with diarrhoea and a negative test for *C. difficile* (non-CDI diarrhoea) and one patient without diarrhoea (non-diarrhoeal). CDI and control patients were matched for hospital, ward and time of diagnosis, which implied selection of control patients that were hospitalized within 14 days of the day on which CDI was diagnosed in the case patient. When several potential control patients were eligible, the first patient on the alphabetical ward list was chosen. A non-CDI diarrhoeal patient was not always available at time of selection. Patients could participate in the study only once.

The Medical Review Ethics Committee of each participating hospital approved the study. No informed consent was required, because only data were used that were available as part of regular patient care. We extracted information on patients' age, sex, co-morbidity and ward of acquisition, previous use of antibiotics (name of drug, dosage, duration of therapy and dispensing dates), co-medication (gastric acid suppressors, non-steroidal anti-inflammatory drugs, immunosuppressive therapy and chemotherapy), admissions and invasive procedures. We used a time period of three months for previous use of medications, admissions and procedures. For CDI patients and for non-CDI diarrhoeal patients, this period was defined as the three months prior to the start of diarrhoea. For non-diarrhoeal patients, we used a three month period prior to a reference date, which was determined by adding the hospitalized period of the matched CDI patient (time between admission and start of diarrhoea) to the admission date of the non-diarrhoeal patient. Using a standardized questionnaire, the data were collected by consulting the physician in charge, using the electronic medical information system and individual patient records. Patients whose records regarding antibiotic use were missing (n=9) were excluded from the study.

Antibiotics were classified into eleven categories (depicted in table 2). The category 'Others' comprised tetracyclines, rifamycins, polymyxins and lipopeptides. We combined the duration and dosage of each prescribed antibiotic by calculation of the Defined Daily Dose (DDD), using a computer tool to calculate antibiotic consumption (ABC Calc 3.1b, available at www.escmid.org/esgap). Co-morbidity was

assessed by both the Charlson Comorbidity Index and the ICD-10 diagnosis, using the tenth revision of the International Classification of Diseases; mentioned in table 1.[17]

Microbiological analysis

Tests for CDI were performed upon request of the physician and on all unformed faecal samples from patients who had been admitted for two or more days, regardless the physicians' request. According to the standard of the local hospital, either one of the following *Clostridium difficile* tests were used: VIDAS *C. difficile* toxin A (bioMerieux), VIDAS *C. difficile* toxin A&B (bioMerieux), Premier *C. difficile* toxins A&B (Meridian), ImmunoCard *C. difficile* (Meridian) or cytotoxicity assay. Toxin positive faecal samples were cultured for the presence of *C. difficile* using a standardized protocol supplied by the Leiden University Medical Center (LUMC). Confirmation of *C. difficile* was performed at the LUMC by the detection of the *glu*D gene.[18] *C. difficile* isolates were further characterized by PCR-ribotyping as previously described.[19]

Statistical analysis

We compared cases to controls without diarrhoea. To determine the period of increased risk for diarrhoea after antibiotic therapy, we also compared cases to non-CDI diarrhoeal patients. We present both comparisons since the results of the first comparison slightly overestimate the effect of antibiotic therapy on the development of CDI and the comparison of cases to non-CDI controls will underestimate this effect, because diarrhoea is a frequent side effect of antibiotic therapy.

Binominal characteristics were compared using the Chi-square test. In all other analyses the individual matching was taken into account. The association between CDI and antibiotic use was analysed using conditional logistic regression, adjusting for age (in 3 categories), sex and Charlson Comorbitidy Index (in 4 categories). In the evaluation of a single antibiotic class this method is referred to as Method 1. Additional adjustments for the use of concomitant antibiotics of different classes were made in the evaluation of a single antibiotic class as a risk factor for CDI by entering all other antibiotic classes into one multivariable model (Method 2). Results are presented as odds ratios (ORs) with the accompanying 95% confidence interval (95% CI). Because we performed concurrent sampling for the selection of controls, the odds ratio is identical to the rate ratio.[20] Statistical significance was reached with a 2-sided p-value < 0.05. We analysed additive interaction between second and third generation cephalosporins and other antimicrobial classes by calculating

the synergy index.[21] We used PASW Statistics version 17.0 (SPSS Inc., Chicago, USA) and STATA software package 10.1 (StataCorp, College Station, USA) for our analyses.

Results

Patient characteristics

A total of 337 CDI patients were included and matched to 337 non-diarrhoeal controls and 227 non-CDI diarrhoeal controls. Clinical and demographical data were complete for the majority of patients (2.7% missing data). Baseline characteristics of included patients are shown in table 1.

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

Patient characteristics	CDI patients (N=337)		Non-diarrhoeal patients (N=337)		Non-CDI patients (N=227)	
Mean age, yr (±SD)	61.8	(±21.1)	59.5	(±21.3)	58.1	(±21.4)
Male sex, no. (%)	184	(54.6)	177	(52.5)	111	(48.9)
Hospital service, no. (%)						
Internal medicine	210	(62.3)	205	(60.8)	156	(68.7)
Surgery	71	(21.1)	78	(23.1)	43	(18.9)
Previous admission, no. (%)	176	(53.8)	97	(29.8)	73	(32.6)
Charlson co-morbidity index, no. (%)						
0	54	(16.2)	68	(20.2)	47	(20.7)
1-2	125	(37.4)	146	(43.3)	88	(38.8)
3-4	102	(30.5)	81	(24.0)	56	(24.7)
5+	53	(15.9)	42	(12.5)	36	(15.9)
Underlying diseases, no. (%) *						
Neoplasms	100	(29.9)	99	(29.5)	69	(30.4)
Respiratory system diseases	81	(24.2)	67	(19.9)	40	(17.6)
Digestive system diseases	91	(27.2)	58	(17.2)	66	(29.1)
Circulatory system diseases	185	(55.1)	170	(50.4)	109	(48.0)
Genitourinary system diseases	119	(35.4)	76	(22.6)	63	(27.8)
Musculoskeletal / connective tissue diseases	42	(12.5)	30	(8.9)	19	(8.4)
Antibiotic therapy, no. (%) **	283	(84.0)	195	(57.9)	132	(58.1)
Immunosuppressive agents, no. (%)	144	(43.4)	115	(34.2)	87	(38.5)
Cytostatic agents, no. (%)	55	(16.5)	39	(11.6)	33	(14.7)

* Underlying diseases were classified according to the tenth edition of the International Classification of Diseases (ICD-10).
** Antibiotic use was defined as the use of any antibiotic during the three-month period prior to the start of diarrhoea or the reference date.

CDI patients had a mean age of 61.8 years, compared to 59.5 and 58.1 years in non-diarrhoeal patients and non-CDI controls, respectively. The CDI patients more frequently had a previous admission to a healthcare facility and more frequently used antibiotics, immunosuppressive and cytostatic agents than non-diarrhoeal controls. All underlying diseases were more prevalent among CDI patients. The prevalence of diseases of the digestive and genitourinary system differed the most, and were present among 27.2% and 35.4% of the CDI patients, and among 17.2% and 22.6% of the non-diarrhoeal patients, respectively (both p<0.01). Non-CDI diarrhoeal patients more frequently had diseases of the digestive system compared to patients with CDI (29.1 versus 27.2 percent; p=0.62).

Table 2. Characteristics of antibiotic use in patients with CDI, control patients without diarrhoea and patients with non-CDI diarrhoea.

Use of antibacterial classes in the 3 months prior to CDI	**CDI patients**		**Non-diarrhoeal patients**		**Non-CDI patients**	
	N=337		*N=337*		*N=227*	
Antibiotic classes, no. patients (%)						
Cephalosporins	185	(56.2)	93	(28.1)	66	(29.3)
1st generation	28	(8.5)	35	(10.6)	12	(5.3)
2nd generation	62	(18.8)	24	(7.3)	26	(11.6)
3rd generation	128	(38.9)	43	(13.0)	41	(18.2)
Penicillins	158	(48.0)	100	(30.2)	78	(34.7)
Fluoroquinolones	89	(27.1)	60	(18.1)	48	(21.3)
Macrolides	17	(5.2)	12	(3.6)	8	(3.6)
Sulphonamides and/or trimethoprim	73	(22.2)	49	(14.8)	44	(19.6)
Aminoglycosides	49	(14.9)	29	(8.8)	31	(13.8)
Carbapenems	21	(6.4)	7	(2.1)	8	(3.6)
Glycopeptides (e.g. vancomycin)	44	(13.4)	24	(7.3)	22	(9.8)
Clindamycin	19	(5.8)	9	(2.7)	12	(5.3)
Metronidazole	53	(16.1)	23	(6.9)	16	(7.1)
Others	27	(8.2)	16	(4.8)	21	(9.3)
Determined within patients with antibiotic use	*N=283*		*N=195*		*N=132*	
No. of antibiotic classes used, mean *	2.68		2.24		2.74	
Time to reference date, geometric mean, days (95% CI) **	3.4	(2.9-3.9)	3.4	(2.8-4.2)	1.9	(1.5-2.4)

* These characteristics were compared using an independent sample t-test.
** Time between the use of the last antibiotic and the start of diarrhoea / reference date; unknown for an additional 35 patients.

Table 3. Crude and adjusted odds ratios of eleven different antibiotic classes as a risk factor for CDI.

	Crude odds ratio (95% CI)	Method 1: Adjusted odds ratio (95% CI)	Method 2: Adjusted odds ratio (95% CI)
Any antibiotic	5.89 (3.57-9.71)	5.84 (3.51-9.70)	N.A.
Cephalosporins			
1st generation	0.77 (0.45-1.32)	0.75 (0.43-1.32)	1.05 (0.48-2.30)
2nd generation	3.47 (1.95-6.16)	3.28 (1.83-5.88)	3.37 (1.61-7.05)
3rd generation	5.53 (3.39-9.01)	5.32 (3.30-8.59)	4.87 (2.80-8.47)
Penicillins	2.41 (1.66-3.50)	2.30 (1.57-3.37)	2.28 (1.43-3.64)
Fluoroquinolones	1.91 (1.24-2.92)	1.82 (1.17-2.83)	0.94 (0.53-1.68)
Macrolides	1.45 (0.68-3.13)	1.31 (0.59-2.93)	0.67 (0.25-1.76)
Sulphonamides and/or trimethoprim	1.81 (1.16-2.83)	1.90 (1.20-3.03)	1.75 (0.98-3.12)
Aminoglycosides	1.86 (1.11-3.13)	1.74 (1.02-2.95)	0.83 (0.42-1.64)
Carbapenems	4.50 (1.52-13.3)	4.70 (1.57-14.1)	5.41 (1.38-21.2)
Glycopeptides (e.g. vancomycin)	2.13 (1.21-3.74)	2.11 (1.18-3.75)	1.05 (0.50-2.21)
Clindamycin	2.25 (0.98-5.17)	2.26 (0.97-5.31)	1.68 (0.58-4.85)
Metronidazole	3.31 (1.78-6.15)	3.35 (1.76-6.37)	2.39 (1.05-5.45)
Others	2.09 (1.02-4.29)	2.07 (0.99-4.32)	1.67 (0.66-4.21)

Each antibiotic class was separately analysed in two multivariable models, adjusting for the variables mentioned in method 1 or 2.
Method 1: corrected for Charlson's index, age and sex (graphically displayed in the online supplementary material).
Method 2: corrected for Charlson's index, age, sex and the use of other antibiotic classes (all classes displayed in the table were separately entered into the multivariable model).
N.A.: not applicable.

Antibiotic agents and the risk for CDI

Type of antibiotic agent – Cephalosporins (mainly cefuroxime and ceftriaxone, both 19%) and penicillins (mainly co-amoxiclav acid, 48%) were the most frequently used antibiotics (table 2). After adjustment for age, sex and Charlson Comorbitidy Index, all antibiotic classes, except 1st generation cephalosporins and macrolides, were associated with CDI (Table 3, 2nd column). Second and third generation cephalosporins and carbapenems had a strong association with CDI: odds ratios of 3.28 (95% CI: 1.83 to 5.88), 5.32 (95% CI: 3.30 to 8.59) and 4.70 (95% CI: 1.57 to 14.1), respectively. Combination therapy of several different antibiotic classes is common in hospitalized patients. We therefore also evaluated the association between antibiotic classes and CDI after adjustment for concomitant use of antibiotics. After these adjustments, confidence intervals overall widened, but second and third generation cephalosporins, penicillins, carbapenems and metronidazole remained significantly associated with CDI (table 3, 3rd column). Furthermore, we performed

an interaction analysis in which no synergistic effect of cephalosporins on any of the other antibiotic classes -or vice versa- was observed (data not shown).

The use of eleven different antibiotic classes of patients with CDI was compared to non-diarrhoeal patients to calculate the strength of the risk of antibiotic use on the development of CDI. This risk was expressed in Odds ratios with a 95% confidence interval. Due to the wide distribution of the effect of cephalosporins, we display three subgroups of cephalosporins separately.

Number of antimicrobials – CDI patients used more different antibiotic classes than non-diarrhoeal controls; a mean of 2.7 versus 2.2 different classes, respectively ($p<0.01$).

Duration and dosage – Figure 1 depicts the effect of dosage and duration (combined in the DDD calculation) of antibiotic therapy on the risk for CDI. This risk increased along with an increasing number of DDDs. The use of ≥14 DDDs of antibiotic in the three months prior to the index date, had the strongest association with CDI (OR 8.50; 95% CI: 4.56 to 15.9).

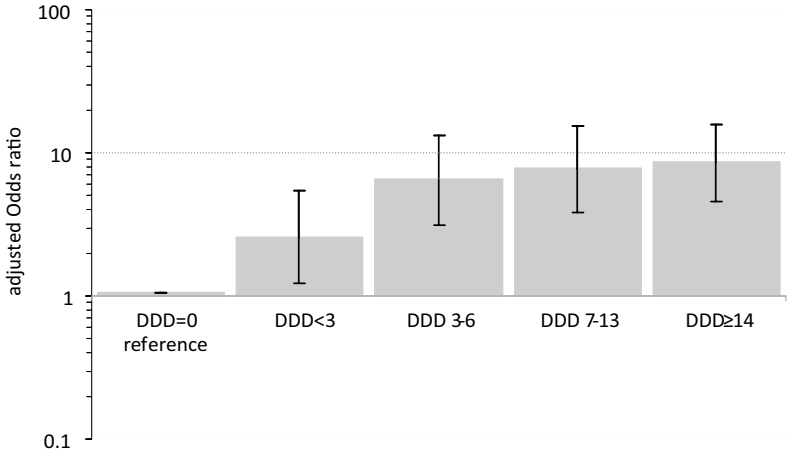


Figure 1. Dose-response relation of antibiotic therapy on the development of CDI. Dose and duration of antibiotic therapy were combined in the calculation of the Defined Daily Dose (DDD). Antibiotic use of CDI cases was compared to that of non-diarrhoeal patients. No use of an antibiotic was used as reference category. Odds ratios were adjusted for Charlson index, sex and age.

Period of increased risk – To determine the time-interval of increased risk for CDI after exposure to antibiotics, we divided the three months prior to the reference date into six intervals (figure 2). In the month prior to the reference date, 242 CDI patients used an antibiotic (76.8%), compared to 157 non-diarrhoeal patients (48.9%)

(p<0.01). Of these, 110 CDI patients (35%) and 80 non-diarrhoeal patients (25%) used an antibiotic at time of diagnosis (p=0.01). Multivariate analysis showed a more than six fold increased risk for CDI during antibiotic use and in the first month after cessation of the antibiotic therapy (OR between 6.67 and 10.37). This risk declined during the period between one and three months after the antibiotic was stopped (OR 2.72; 95% CI: 1.20 to 6.15). Additionally, we displayed the comparison of CDI patients versus non-CDI diarrhoeal patients in figure 2. This comparison also showed an increased risk for CDI in the first month after cessation of antibiotic therapy (OR between 5.24 and 9.35). When an antibiotic was used at the start of diarrhoea, the risk for CDI was lower (OR 2.41; 95% CI 1.30 to 4.46), which can be explained by the occurrence of antibiotic associated diarrhoea in non-CDI diarrhoeal patients.

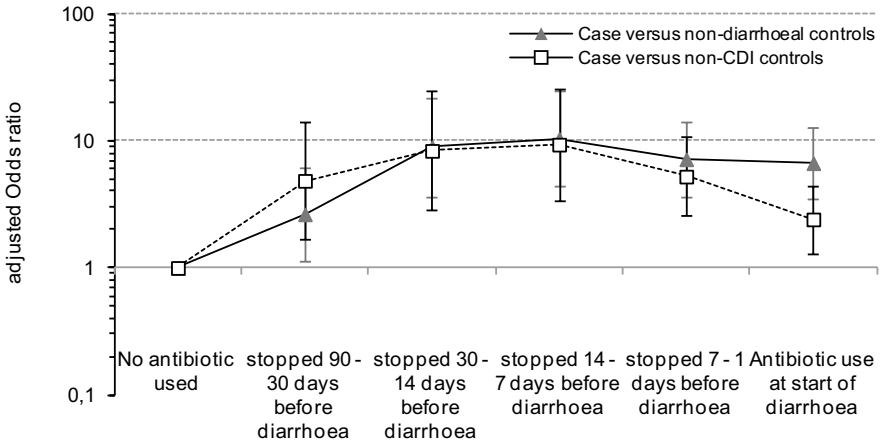


Figure 2. The period at risk for CDI after cessation of antibiotic therapy. The use of antibiotics of patients with CDI compared to non-diarrhoeal patients and patients with non-CDI diarrhoea, stratified in six time intervals. This was done to calculate the risk for CDI after cessation of antibiotic therapy. The Odds ratio was adjusted for age, sex and Charlson index.

Microbiological characteristics

Isolates from 211 (58%) CDI patients were available for further characterization. In 192 (91%) of these, we were able to perform PCR ribotyping. Type 014 was the most frequently found type (n=34; 18%), followed by type 078 (n=24; 13%), type 001 (n=17; 9%) and 027 (n=16; 8%).

Discussion

In this multicenter case-control study, we analysed the period of increased risk for CDI after antibiotic therapy. We found a seven to ten fold increased risk for CDI during antibiotic therapy and the first month after cessation of antibiotics. Another important finding of our study was that antibiotic use one to three months before development of diarrhoea could still be associated with CDI. Second and third generation cephalosporins and carbapenems were the most potent risk factors. The risk for CDI increased when a larger amount of antibiotics and more antibiotic classes were used.

Our findings regarding the time-interval of increased risk are in accordance with the results of a previous study that investigated a specific patient population of elderly patients, who were admitted due to severe community-acquired CDI.[14] The generalizability of this Canadian study was however limited, because it comprised only a small fraction of the patient population that was included in our study. The period of increased risk also coincided with changes in the gut microbiota that occur within days after the start of antibiotic therapy and can persist for weeks or even years after cessation of the antibiotic.[22, 23] Because the intact commensal bowel flora protects against intestinal colonization and infection by *C. difficile*, disruption of the flora during and after antibiotic therapy can result in outgrowth and toxin production of *C. difficile*.[24]

The duration of therapy and dosage of antibiotics, expressed as DDD, showed a positive correlation with the risk of CDI, which is in line with previous reports, as well our finding that virtually all antibiotic classes were associated with CDI.[8, 25, 7] In the literature, fluoroquinolones have mainly been associated with CDI due to PCR ribotype 027.[25] Because we encountered this type in only 8% of our patients, this antibiotic class was not among the most potent risk factors in our study. First generation cephalosporins, which are regularly used as a perioperative prophylaxis, were not associated with CDI in our analyses. This is in line with previous studies, where this antibiotic class was associated with a relatively small risk, or even a decreased risk, on the development of CDI. [8, 25, 26] The latter was suggested to be a result of not severely ill patients with short admissions who received small amounts of first generation cephalosporins. Because cases and controls in our study were selected from the same department and patients receiving a first generation cephalosporin did not represent a specific population (same age and Charlson comorbidity index as patients not receiving this cephalosporin), we assume that

first generation cephalosporins affect the gut microbiota to a lesser extent and do not increase the risk for development of CDI. Administration of metronidazole or vancomycin has infrequently been associated with an increased risk for CDI.[8, 25] In the present study, most patients received intravenous metronidazole or vancomycin for systemic treatment of infections other than CDI, but 6.5% of the patients were treated orally. After excluding these patients, metronidazole and vancomycin remained associated with CDI but the association became weaker (adjusted ORs 3.08 and 1.68 for metronidazole and vancomycin, respectively, according to method 1).

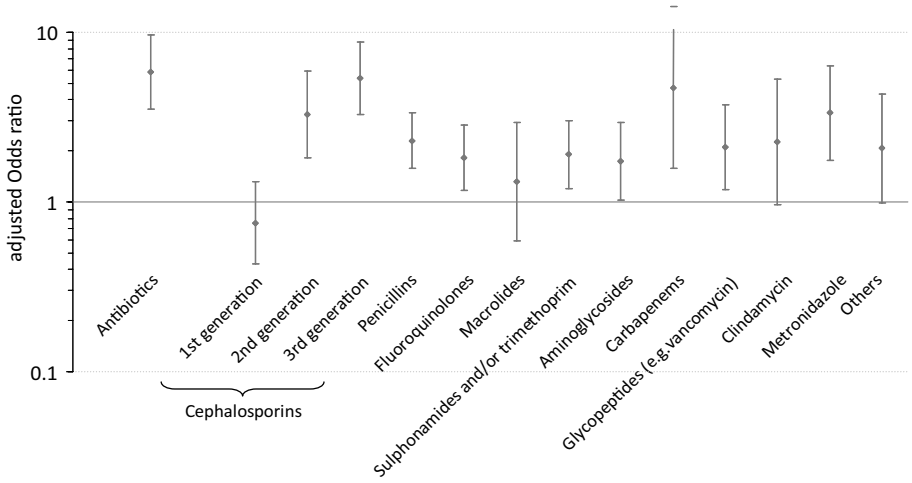
One approach to analyse the risk for CDI associated with a certain antibiotic class, is to restrict the analysis to cases and controls not using other antibiotics. Since only a minority of our CDI patients used antibiotic monotherapy (n=36; 11%), this approach was not feasible. We therefore analysed the effect of a single class of antibiotics by including all cases and controls and adjusting in a logistic model for the use of concomitant antibiotic classes. The advantage is an increased power of the analyses because all cases and controls are included. The estimated Odds ratios will be valid provided that confounding will be adequately adjusted for, a condition that cannot be proven empirically.[27] Confounding was, however, minimised by adjusting for all antibiotic classes.

The most important strength of this study is the robustness of the dataset that was generated by combining data from electronic medical systems, patient records and direct consultation of the physician. Furthermore, we reduced ascertainment bias by testing all unformed stool samples, irrespective of the physician's request. Matching CDI patients and their controls on ward and time of admission ensured us that these patients originated from a setting with a comparable CDI pressure, which has been described as an important risk factor for CDI.[28] Finally, our results are applicable to non-outbreak situations, since the study was performed in a setting in which multiple PCR ribotypes caused CDI (39 different types).

A limitation of our study is the use of various enzyme immuno assays to diagnose CDI. The reported sensitivity of these tests varies between 60% and 85%.[29, 30] Therefore, patients in our study could have been missed as patients with CDI. Consequently, the time of increased risk of non-CDI diarrhoea after antibiotic use might have been overestimated. A second limitation of our study is the use of two control groups. About ten percent of the patients admitted to a (university) hospital experience diarrhoea during their admission. Therefore, a control group that would have been selected without considering the presence of diarrhoea would have been more representative.[31, 32] Analysis of our data after combining the control groups

of patients with non-CDI diarrhoea and non-diarrhoeal patients did however not influence our conclusions (data not shown).

In conclusion, the interval of increased risk for CDI after antibiotic therapy comprises the time from the actual antibiotic use until three months thereafter. The highest risk for CDI is found during and in the first month after antibiotic use. Clinicians should be aware that antibiotic use can increase the risk for CDI a tenfold, even if the antibiotic use preceded the symptoms by one month. Additionally, the results of our study could help future researchers to more accurately define the period of increased risk for CDI after antibiotic exposure.



Supplementary figure. The Odds ratios of eleven different antibiotic classes as a risk factor for CDI. The use of eleven different antibiotic classes of patients with CDI was compared to non-diarrhoeal patients to calculate the strength of the risk of antibiotic use on the development of CDI. This risk was expressed in Odds ratios, using a confined correction method, correcting for Charlson index, age and sex. Odds ratios are displayed with a 95% confidence interval. Due to the wide distribution of the effect of cephalosporins, we display three subgroups of cephalosporins separately. Absolute numbers are displayed in Table 3, 2nd column.

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Chapter 6

Diarrhoea in general practice:

when should a *Clostridium difficile* infection be considered?

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Submitted

Abstract

Objective To determine the incidence of *Clostridium difficile* infections in general practice. To evaluate current testing algorithms and develop a score that predicts the pretest probability of CDI in the community and use this score to guide diagnostic testing.

Design Prospective cohort study (incidence determination) with nested case control study (prediction score).

Setting Three Dutch laboratories tested all unformed faeces of patients ≥2 years for whom diagnostic testing (for any enteric pathogen) was requested by a general practitioner between October 2010 and January 2012.

Participants The three laboratories serviced a total of 2,810,830 patients, of whom 12,714 faecal samples were submitted for diagnostics. 152 patients with a positive test for *C. difficile* toxin and 304 age and sex matched controls with a negative test participated in the case control study.

Main outcome measures The incidence of *C. difficile* infection (CDI) was calculated using the general practitioners patients list. Weighted multivariable logistic regression was used to compare CDI patients to controls and to construct a prediction score. Its performance was compared to other testing regimens by calculating the percentage of diarrhoeal patients that require testing and the percentage of CDI that is detected by following this regimen.

Results 194 of 12,714 unformed stool samples (1.5%) were positive for *C. difficile* (incidence 0.67 per 10,000 patient years). This incidence was lower than that of *Campylobacter*, but comparable to the incidence of *Salmonella*. Compared to diarrhoeal controls, CDI was associated with more severe complaints, underlying diseases, antibiotic use and prior hospitalization. After multivariable analysis, a prediction score consisting of 7 clinical parameters and good calibration and discrimination (ROC 0.79) was constructed. Testing unformed stool samples of patients with a prediction score of ≥3 (44% of samples), would result in detection of 85% of CDI in general practice. In our study, general practitioners requested a test for *C. difficile* in 7% of the stool samples, hereby detecting 40% of all CDI. Dutch national recommendations advise general practitioners to test for *C. difficile* when prior antibiotic use or hospitalization is present (18% of samples). If these recommendations were followed, 61% of all CDI would have been detected.

Conclusion *C. difficile* is relatively frequent in diarrhoeal stool samples from general practice. Currently, testing for *C. difficile* is rare and only 40% of CDI in general practice is detected. Following recommendations that are based on traditional risk factors for CDI, would improve detection of CDI to 61%. We propose a clinical prediction score to guide testing which detects 85% of the CDI patients, without testing a large number of samples.

Introduction

Clostridium difficile infection (CDI) is a common cause of hospital-acquired diarrhoea. Elderly patients with underlying diseases and recent antibiotic therapy are primarily affected, resulting in prolonged hospitalization and excess mortality[1]. Recently, CDI was reported as an emerging disease outside healthcare facilities[2]. More than a quarter of all CDI is estimated to be currently acquired in the community[3]. In contrast to nosocomial CDI, patients in the community are younger, antibiotics are less frequently used and routes of exposure are often unknown. Consequently, over a third of these patients has no known risk factors for CDI[4, 5]. This makes recognition of CDI problematic; especially since *C. difficile* is not widely tested for in general practitioners' practice.

In 2009 a guideline was introduced in the UK, stating that all cases of diarrhoea among patients ≥2 years in the community should be tested for *C. difficile* unless good clinical or epidemiological reasons not to, are present[6]. Diarrhoea is common in general practice, reaching incidences of 200 per 10,000 person years[7, 8], which makes comprehensive testing costly. Consequently, the UK guideline was adapted in 2012 and advised to test all diarrhoeal samples of elderly patients or patients with risk factors[9]. In most countries, including the Netherlands and the USA, guidelines for general practitioners still state that *C. difficile* should be suspected in patients with a recent hospitalization or antibiotic use[10, 11], which may result in missed diagnoses.

Although the need to characterize patients with CDI in the community is high, few studies focused on clinical presentation and additional characteristics of this patient group[5]. Additionally, studies often select diagnosed (and therefore recognized) patients only. Therefore, we decided to describe the occurrence of CDI in a laboratory-based cohort study, testing for *C. difficile* irrespective of whether the general practitioner requested *C. difficile* testing. Using this design, we aimed to determine the incidence of toxigenic *C. difficile* and to predict which patients have CDI. Additionally, we used these predictions to evaluate and guide current diagnostic algorithms.

Methods

Study design

The study was set in three medical microbiological laboratories: Stichting Huisartsen Laboratorium (Etten-Leur), the Laboratory for Medical Microbiology

and Immunology of the St. Elisabeth hospital (Tilburg) and the Laboratory for Clinical Microbiology and Infectious diseases of the Isala klinieken (Zwolle). These laboratories supply microbiological services to 832 general practices with together 2,810,830 patients. Between October 4th 2010 and January 31st 2012, all unformed stool samples of patients ≥2 years, submitted by general practitioners (GPs), were prospectively tested for the presence of *C. difficile* toxin irrespective of whether the GP requested testing for *C. difficile*. Samples were excluded when a patient (1) had a prior positive test or (2) was tested within the previous 30 days. Unformed stool was defined as 'taking the shape of the container'[12].

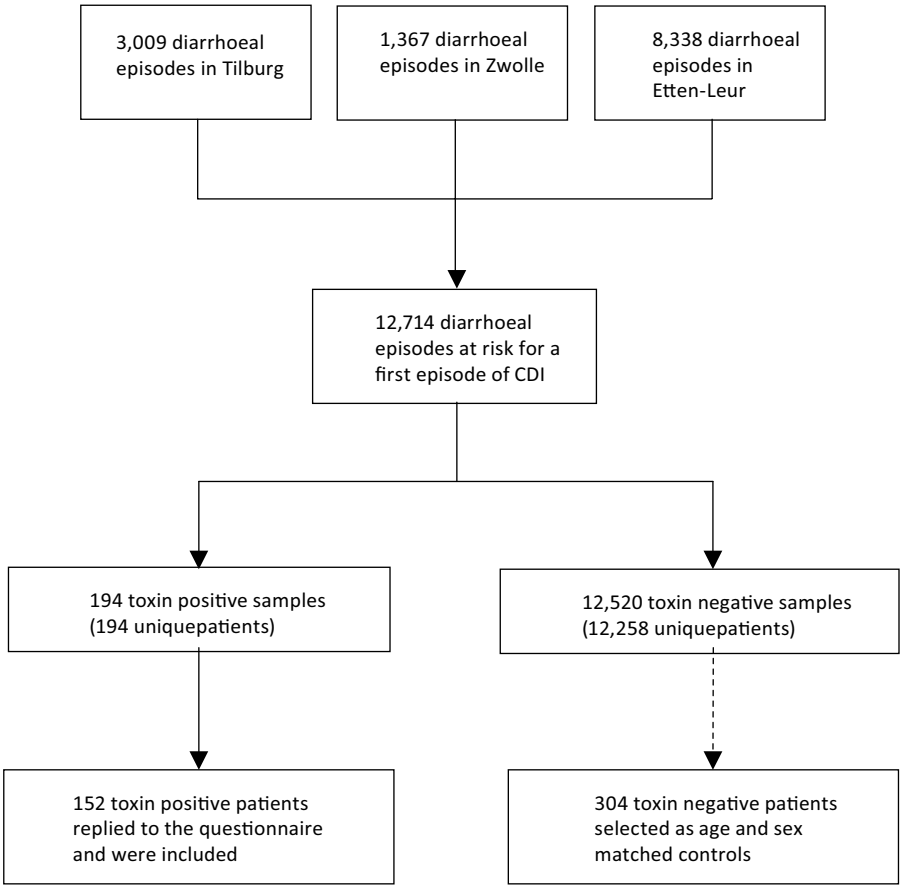

Figure 1. Patient inclusion chart.

Patients with a positive test for *C. difficile* toxin were defined as CDI. Using a nested case-control design, patients with CDI were matched on age (±5 years) and sex to two control patients. Control patients were selected from the cohort of toxin

negative patients and tested negative at most one week before the case. If a control patient was not available at that time, the first patient after the index date (date of CDI case) was selected. The study protocol was approved by the LUMC Medical Review Ethics Committee.

Definitions and data collection

Of all tested patients we collected basic demographic data. One laboratory (Etten-Leur) additionally registered if the *C. difficile* test was specifically requested by the GP. This was used to evaluate whether CDI testing was requested in current practice.

After obtaining permission of the GP, questionnaires were sent to CDI patients and sampled controls. Either via the GP or by contacting the patient directly by mail or telephone, we requested the questionnaire; this was done up to six times. Questions focused on medication and contact with infants or healthcare in the three months before diarrhoea, co-morbidity in the year before diarrhoea, travelling history and proximity to other patients with diarrhoea. Frequency, viscosity and presence of bloody diarrhoea were ascertained at the height of the complaints of the diarrhoeal episode. All variables, except for abdominal pain and fever, which were deemed too subjective, were considered potential predictors for CDI diagnosis. Follow-up of patients with CDI was done after six months by contacting the GP and informing on the initiated treatment for CDI and presence of relapses or death.

Stool examinations

The presence of toxin producing *C. difficile* was assessed by a cell cytotoxicity assay in Tilburg, which is still regarded as the reference standard[13, 14]. The two other laboratories used an enzyme immunoassay (EIA) for toxins A and B (Premier toxins A&B, Meridian).

Upon request of the general practitioner, faeces was tested for diarrhoeal pathogens other than *C. difficile*. These pathogens were tested using local available tests (all PCR). Testing was possible for: bacterial pathogens (*Salmonella* spp., *Shigella* spp. and *Campylobacter* jejuni/coli), parasitic pathogens (*Cryptosporidium*, *Giardia lamblia* and *Entamoeba histolytica*) or viruses (norovirus) in all three laboratories. Additional tests were available upon request or if deemed clinically relevant based on patient data (data not shown). All microbiological results, including the result of the *C. difficile* toxin test, were reported to the GP.

Stool samples that were positive for *C. difficile* in the initial test, were cultured and isolates were typed with PCR ribotyping[15]. When an isolate could not be

obtained, a PCR on the *tcdB* gene was performed on faeces to confirm the presence of toxigenic *C. difficile*[16].

Data analysis

Incidence rates of diarrhoea and intestinal pathogens were calculated using the total number of person years at risk, which was calculated by multiplying the general practice population (the number of people serviced by the participating general practitioners, according to their patient list) and the period of study participation (between 12 and 15 months).

CDI patients and matched controls were compared with univariate conditional logistic regression. Results were displayed as odds ratios (OR) with a 95% confidence interval (95% CI). Subsequently, variables with a p-value of <0.10 were included in a multivariable model. Although these variables were complete in >92% of the CDI patients and controls, we used multiple imputation to account for missing values in multivariate analysis. This method is appropriate when predictors of the missing data are available (missing at random; MAR)[17]. All potential predictors of missing data, potential predictors of the outcome and the outcome itself were included in the imputation procedure. To predict the absolute risk of CDI and to include the matched variables (age and gender) in the multivariate analysis, we performed case-control weighting[18]. This was possible due to the fact that the case control study was nested in a cohort. Weights were determined by prevalence, age and sex distribution of cases and controls compared to the original cohort. In cases, weights varied only marginally (between 1.2 and 1.4), since 78% of the diagnosed CDI patients participated in the case control study. Weights of controls varied between 17 and 112 (mean 41), emphasizing the large sampling fraction and the relative overrepresentation of elderly patients due to matching. Using stepwise backward regression (p>0.25 based on the likelihood ratio test) we selected the strongest predictors in the weighted multivariable model. To construct a prediction score, we rounded the regression coefficients of the predictors to the nearest half and doubled them to construct integers (i.e.0.25 to 0.75 was 1; 0.75 to 1.25 was 2; and so on). Discrimination of the score was evaluated by calculating the area under the Receiver Operating Curve (ROC area). Calibration of the score was assessed by comparing the predicted and observed probability to have CDI. For several cut-off points we determined the performance of the score: the percentage of tests required, the percentage of positive results and the percentage of detected CDI.

The performance of the score was compared to (1) current diagnostic practice of general practitioners by evaluating the samples in which the GP requested testing for *C. difficile*. To compare the performance of the score to (2) the current advice in the Netherlands, (3) the current advice in the UK and (4) the former advice in the UK, the weighted cases and controls were used. We calculated the percentage of diarrhoeal patients that required testing according to the aforementioned advices, by calculating the prevalence of clinical characteristics in our weighted population of diarrhoeal cases and controls (e.g. prevalence of patients with antibiotic use or prior admission was calculated, because these patients require *C. difficile* testing according to current Dutch recommendations). In the population that required testing, we determined the percentage of CDI (e.g. among patients with prior antibiotic use or admission, 8% was CDI positive). Additionally, we determined the percentage of CDI patients that would have been tested by the algorithm (e.g. 60% of all CDI patients occurred in the in the group of patients with prior antibiotic use or an admission).

We used SPSS version 20.0 (SPSS Inc., Chicago) and STATA software package 10.1 (StataCorp, College Station) for our analyses.

Table 1. Age, gender and incidence of intestinal pathogens in unformed stool samples with a test request from the general practitioner.

	Samples (N=12714)				
	no. of cases	**% of all samples**	**rate per 10,000 person years (95% CI)**		**no. of samples tested**
Female gender	7302	57.4%			
Age, mean (±sd)	41.3	(23.2)			
Pathogen detected	2786	21.9%	9.68	(9.33-10.05)	12566
Diagnosed pathogens					
Campylobacter coli / jejuni	1056	8.3%	3.67	(3.45-3.90)	10598
Giardia lamblia	454	3.6%	1.58	(1.44-1.73)	8954
Salmonella spp.	198	1.6%	0.69	(0.60-0.79)	10598
Clostridium difficile	194	1.5%	0.67	(0.58-0.78)	12714
Shigella spp.	114	0.9%	0.40	(0.33-0.47)	10598
Cryptosporidium	107	0.8%	0.37	(0.31-0.45)	8954
Norovirus	75	0.6%	0.26	(0.21-0.32)	1374
Entamoeba histolytica	2	0.0%	0.01	(0.00-0.02)	6720

All samples were tested for *C. difficile*, whereas other pathogens were tested upon request of the general practitioner. All laboratories used a PCR to detect the pathogens: *Campylobacter*[33, 34], *Salmonella*[34, 35], *Shigella*[36], *Giardia lamblia*[37-39], *Cryptosporidium*[37, 38], *Entamoeba histolytica*[37, 38], Norovirus[40].

Results

During the study period, 12,714 unformed stool samples met the study's inclusion criteria (Figure 1). The incidence of diarrhoea in which faeces investigation was requested was 44 per 10,000 person years. Patients were on average 41.3 years old and the majority was female (57.4%) (Table 1).

Incidence of *C. difficile* infection

Of 12,714 stool samples, 194 (1.5%) were positive for *C. difficile* (incidence 0.67 per 10,000 patient years). In Tilburg a cell cytotoxicity assay (considered as reference standard) was used to diagnose CDI. Here, 54 tests were found positive among 3009 diarrhoeal samples (1.8%; 1,03 per 10,000 patient years).

99% of the stool samples were also tested for the presence of pathogens other than *C. difficile* (12566/12714), which was identified in 21.9% (2786/12714) of all samples: in 22.1% of the CDI negative samples (2763/12520) and in 11.8% (23/194) of the CDI positive samples. The most frequently found co-pathogen in CDI positive samples was *Campylobacter coli / jejuni* (n=10; 5%). *Campylobacter coli / jejuni*. and *Giardia lamblia* were found in 8.3% (1056/12714; 3.67 per 10,000 person years) and 3.6% (454/12714; 1.58 per 10,000 person years) of all samples, respectively. *Salmonella* spp. was found in percentages similar to *C. difficile*: 1.6% (198/12714; 0.69 per 10,000 person years).

CDI vs controls with diarrhoea

Within the cohort of 12,714 samples, we performed a nested case-control study. 152 of 194 CDI patients (78%) completed the questionnaire and were matched on age and gender to 304 controls. Participating CDI patients were on average 52.3 years old (standard deviation 22.5), 61% was female. Symptoms of diarrhoea started in the community in 94% (n=143). Three patients (2%) developed symptoms in a long term care facility and six (4%) developed diarrhoea during hospitalization but were diagnosed after discharge. Compared to controls, CDI patients more often had severe symptoms (bloody stools, watery or frequent diarrhoea), underlying diseases, prior hospitalization and prior use of antibiotics (univariate analysis; Table 2). A third of the CDI patients (n=58; 39%) did not use antibiotics nor was previous hospitalized; 14% of the CDI patients (n=22) had no underlying diseases, hospitalization or medication use prior to diarrhoea. CDI patients reported abdominal pain and fever in 77% and 31%, respectively; controls reported these symptoms in 75% and 20%, respectively.

Table 2. Clinical characteristics of CDI patients and matched control patients, analysed with conditional logistic regression analysis.

Possible predictors	CDI Cases (N=152)		Controls (N=304)		Crude analysis		
	N	*%*	*N*	*%*	*OR*	*95% CI*	*p-value*
Symptoms							
Bloody stools	36	25.2	44	15.7	1.82	1.07-3.09	0.03
Watery diarrhoea	119	78.3	207	68.1	1.71	1.08-2.71	0.02
Frequency of diarrhoea >8 times	68	44.7	75	24.9	2.39	1.59-3.61	<0.01
Time to visit GP <1 month	96	64.5	165	56.3	1.40	0.94-2.10	0.10
Medication							
Antibiotics	82	55.0	49	16.6	8.15	4.57-15.5	<0.01
Other medication	92	60.5	166	56.1	1.26	0.81-1.98	0.31
PPI / antacida	43	29.1	60	21.1	1.59	0.99-2.55	0.06
Statin	25	16.9	40	14.1	1.38	0.74-2.58	0.31
NSAID	11	7.4	24	8.4	0.80	0.37-1.73	0.57
DM	10	6.8	19	6.7	1.03	0.46-2.28	0.95
Immuunsuppression	11	7.4	12	4.2	1.72	0.74-4.02	0.21
Diuretics, antihypertensives	47	30.9	76	25.2	1.48	0.87-2.53	0.15
Underlying diseases							
Any disease	90	59.2	120	39.7	2.64	1.66-4.20	<0.01
Circulatory system diseases	18	11.8	34	11.3	1.09	0.54-2.19	0.81
Respiratory system diseases	24	15.8	26	8.6	1.90	1.08-3.36	0.03
Cancer	10	6.6	7	2.3	3.60	1.21-10.7	0.02
Environment							
Previous admission	28	18.4	21	7.0	3.16	1.67-5.99	<0.01
Family member with diarrhoea	7	4.8	23	8.0	0.58	0.25-1.35	0.20
Infant <2 year old	40	27.6	97	32.2	0.75	0.47-1.20	0.23
Visit foreign country							
In western world	16	15.4	43	18.4	0.79	0.40-1.56	0.50
Outside western world	15	14.4	41	17.5	0.77	0.38-1.58	0.48

The crude analysis was done by univariate conditional logistic regression, which takes in account the matched factors 'age' and 'gender'. Variables with a p-value <0.10 (n=11) supplemented with age and sex were included in the multivariate analysis (table 3).

Predicting CDI

Nine variables had a p-value of <0.10 in univariate analysis and were included in multivariate analysis, together with age and gender. After backward regression, seven variables remained in the model and were included in a prediction score. Age ≥50 years, watery diarrhoea, an underlying disease in the year before start of diarrhoea and hospitalization in the preceding 3 months were strong predictors for CDI and received a score of 1 point. Frequent diarrhoea (>8 times daily) and cancer in the preceding year were scored as 2 points and antibiotic use in the preceding

three months as 4 points (Table 3). For each patient a score was calculated based on the sum of these points (minimum 0 to maximum 12 points). Predicted and observed probabilities to have CDI were similar in the far majority of the patients (Table 4). The ROC area of the score for the detection of *C. difficile* was 0.79. Adding all variables with a p<0.10 in univariate analysis to the model did not improve model and yielded a similar ROC area (0.79).

Table 3. Multivariable analysis of potential predictors using weighted logistic regression analysis and the construction of a risk score.

Predictors	Full MVA			Restricted MVA			
	OR	*95% CI*	*p-value*	*OR*	*95% CI*	*Regression coefficient*	*Score*
Age ≥ 50	1.41	0.79-2.52	0.25	1.36	0.80-2.32	0.31	1
Gender	1.18	0.70-1.99	0.53				
Bloody stools	1.16	0.60-2.25	0.65				
Watery diarrhoea	1.55	0.86-2.81	0.15	1.50	0.84-2.68	0.41	1
Frequency of diarrhoea >8 times	2.87	1.66-4.96	<0.01	2.90	2.69-4.99	1.07	2
Antibiotics	6.88	3.97-11.9	<0.01	7.26	4.29-12.3	1.98	4
PPI / antacida	1.10	0.56-2.08	0.77				
Any disease	1.80	1.00-3.23	0.05	2.00	1.19-3.33	0.69	1
Respiratory system diseases	1.25	0.51-3.06	0.63				
Cancer	4.04	1.47-11.1	<0.01	3.45	1.33-8.93	1.24	2
Previous admission	1.66	0.75-3.68	0.21	1.63	0.76-3.53	0.49	1

In the 'full multivariate analysis (MVA)' we included all variables with a p-value of <0.10 according to the crude analysis, 'age' and 'gender'. Using backward regression analysis, the strongest predictors were selected (p<0.25; n=7). Subsequently, a score was assigned to the selected variables, based on the regression coefficient. Both MVAs used weighted CDI patients and controls (see methods).
To calculate the probability than an individual patient has CDI, the following formula can be used: p = 1 / (1 + exp -(-6.06 + 0.31*age ≥50 + 1.07*frequency of diarrhoea >8 times + 1.98*antibiotics + 0.69*underlying diseases + 1.24*cancer + 0.41*watery diarrhoea + 0.49*previous admission)).

Performance of testing algorithms

The prediction score developed, enables selection of patients at high risk for CDI. Consequently, this score might guide testing for *C. difficile*. We present cut-off points that are suitable for three aims: (1) detecting a large number of CDI patients, (2) testing a minimum number of diarrhoeal stool samples, (3) a combination of these aims. Based on the weighted case-control data, a cut-off point of ≥3 to test a patients' faeces for *C. difficile*, would result in testing 44% of all diarrhoeal stool samples and detecting 85% of all CDI patients. A cut-off of ≥8 would require testing 2% of all diarrhoeal samples and detecting a quarter of all CDI (27%). A balanced cut-

off point was set at ≥6, where 11% of all diarrhoeal would need testing and 50% of all CDI is detected (Table 5).

Table 4. Calibration of the risk score that can be used to predict a *C. difficile* infection.

	Patients N=12714		**Observed probability**	**Predicted probability**
Score	*(n)*	*(%)*	*(%)*	*(%)*
0	1730	13.61%	0.20%	0.23%
1	2895	22.77%	0.43%	0.37%
2	2488	19.57%	0.50%	0.58%
3	1889	14.86%	1.57%	0.99%
4	1376	10.82%	1.55%	1.61%
5	874	6.88%	2.02%	2.60%
6	723	5.68%	3.06%	4.33%
7	459	3.61%	5.12%	6.81%
8	166	1.30%	15.71%	9.96%
9	80	0.63%	15.91%	17.19%
10	31	0.24%	29.01%	25.65%
11	3	0.02%	100.00%	41.10%
12	1	0.01%	100.00%	53.25%
Total	**12714**	**100.00%**		

In this table, we display the prevalence of each level of the risk score. Furthermore, we show the observed and predicted probability on CDI.

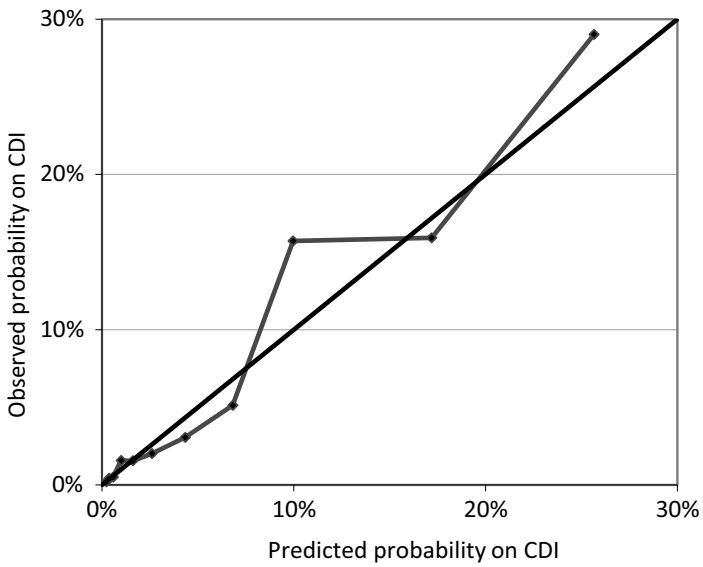

Table 5. Performance of seven different algorithms for testing diarrhoeal samples on *C. difficile* in general practice.

		Patients tested	Positive results	Detection of CDI
Test algorithm for CDI in diarrhoeal samples from the community	**Setting**	*% of all unformed stool samples*	*% of all tested samples*	*% of all positives*
≥ 2 years	former advice UK (2009)	100%	1.5%	100%
≥ 65 years, after AB use or hospitalization	current advice UK (2012)	31%	3.5%	72%
After AB use or hospitalization	current advice NL	18%	5.0%	61%
Docter's current practice	current practice NL	7%	8.1%	40%
Prediction rule, cut-off ≥3	this study	44%	2.9%	85%
Prediction rule, cut-off ≥6	this study	11%	6.7%	51%
Prediction rule, cut-off ≥8	this study	2%	18.4%	27%

These percentages are based on the weighted analysis of all CDI patients and controls (n=12714).

According to data from one laboratory (Etten-Leur), general practitioners request a test for CDI in 7% of submitted samples (543/8338). These samples included 40% of all diagnosed CDI patients in this study. Currently, the advice to test for *C. difficile* in general practice in the Netherlands is to test all patients with diarrhoea and recent antibiotic use or hospitalization. As 18% of the patients in the study recently used antibiotics or were hospitalized, this advice would lead to testing of 18% of all diarrhoeal patients, detecting 61% of all CDI patients. In the United Kingdom, all diarrhoeal patients aged ≥65 years or patients with recent antibiotic use or a recent hospitalization are advised to be tested. Implementing his strategy in our study population would result in detection of two-thirds of all CDI patients, whereas it would require testing 31% of all diarrhoeal samples.

Confirmation of *C. difficile*

Of the 152 cases with CDI, the presence of *C. difficile* could be confirmed by PCR ribotyping or a positive *tcdB* PCR in 68% (n=103): types 002 and 078 (both n=11; 11%) were most frequently found; type 001 (8%), 005 (6%), 014 (8%), 015 (9%) and 126 (4%) were other frequently found PCR ribotypes. The virulent type 027 that caused many outbreaks in hospitals[19], was isolated in one patient with frequent relapses and prior long term hospitalization. Thirty-five stool samples were not available for confirmation testing with the *tcd*B PCR. The majority of the CDI patients in the case control study had *C. difficile* as the only detected pathogen (130 of 152; 86%).

Six months follow-up

Of 122 CDI patients with known follow-up (80.3%), the majority (n=96; 78.7%) was treated for the infection: monotherapy with metronidazole was most frequently used (n=85; 88.5%), 6 patients were treated with vancomycin (6.3%), 3 with a combination of both (3.1%). Thirty patients (24.6%) had recurrent diarrhoea within 6 months, which was confirmed by a positive toxin test in 36.7%. Within 6 months, 6 CDI patients (3.9%) were hospitalized because of diarrhoea and 4 died (2.6%). In one patient (0.6%) CDI contributed to the cause of death.

Discussion

Patient Example

A 68 year old woman with diabetes type II, hypertension visits her general practitioner with complaints of acute watery diarrhoea, that occurred more than 10 times daily. She did not use any antibiotics in the prior 3 months and was not hospitalized in the prior 3 months, but recovered from breast cancer 9 months ago. The doctor requests microbiological examination of a stool test to test for the presence of *Salmonella*, *Shigella* and *Campylobacter*. After reading this publication he wonders: should I think of *C. difficile* as well? Based on the prediction score we presented here, his patient has a summed score of 7 points (age>50 years = 1 point; underlying diseases = 1 point; watery diarrhoea = 1 point; a frequency of more than 8 times daily = 2 points; cancer = 2 points). According to Table 4 (based on the formula below Table 3), the probability to have CDI for this woman is 6.8%. The doctor decides to request a test for *C. difficile*.

Incidence of CDI in general practice

This study determined the incidence of *C. difficile* in a large sample of diagnostic test requests from general practitioners. One out of 66 diarrhoeal episodes was positive for *C. difficile* (1.5%), which was comparable to the incidence of *Salmonella* spp.. Earlier studies reported similar incidences of CDI (1.5 to 2.1%[4, 5]; 0.7-2.5 per 10,000 person years[2, 5, 8, 20-25]), with the exception of a study from the UK that reported virtually no CDI in general practice[26]. The latter UK study confirmed our relatively low rate of salmonellosis (1.8 per 10,000 patient years using faecal culture), but should be interpreted with caution since exclusion criteria such as recent travel and diarrhoeal illness lasting over 2 weeks resulted in the analysis of 45% (991/2203) of all diarrhoeal episodes. Although we included all diarrhoeal samples that were

sent to a laboratory, the incidence of CDI in our study could be underestimated if diarrhoeal samples of patients with CDI were not sent to a laboratory and the disease had a self-limiting course.

Our study included 12,714 diarrhoeal episodes and showed that CDI is relatively common among diarrhoeal stool samples and should be included in the differential diagnosis of infectious diarrhoea in general practice.

When should we consider CDI and request a test?

Dutch GPs are recommended to test all patients with prior antibiotic use or hospitalization for CDI. Currently, GPs do not follow these recommendations and test only 7% of all diarrhoeal patients, detecting 40% of all CDI patients. This large proportion of undiagnosed patients with CDI is in our opinion undesirable, as all CDI patients had diarrhoeal complaints and nine patients (5.8%) experienced a complicated course (hospitalization or death within 6 months). A similar course was observed in community-based studies[5, 24], however, as most CDI patients in these studies were treated for CDI, we expect the number of complicated courses to be higher when CDI is undiagnosed and therefore untreated. In our study, complicated courses were also experienced by patients without traditional risk factors (3/9; 33.3%), which underlines the necessity of diagnosis.

Because testing of all samples, as was the former UK advice, requires a large budget, this is currently probably not achievable in most laboratories and general practices. Our study confirms that well known risk factors for nosocomial CDI, antibiotic use or hospitalization, are present in only 61% of the patients with CDI in the community. To distinguish CDI from other causes of diarrhoea we constructed a prediction score in which disease symptoms and underlying diseases were good predictors besides age, previous antibiotic use and hospitalization. This enables the physician to estimate the risk for CDI (see Patient Example) and use this to consider testing. According to our study, testing all patients with a prediction score of ≥3 would be a cost saving option, compared to testing all patients, that still detects 85% of all CDI. Although we would like to pose this prediction score as a guide for *C. difficile* testing due to good calibration and discrimination, its performance should be confirmed during external validation in a different setting before wide implementation in clinical practice. Until this is done, we recommend to follow current Dutch guidelines or the current UK advice in the Netherlands. This would result in detection of 61% or 72% of all CDI, respectively, which would clearly outperform current practice.

Strengths and weaknesses

We are the first to provide a complete overview of incidence, clinical characteristics and testing strategies of CDI in general practice. The size of the cohort and high participation rate (78%), ensuing the early and thorough follow-up of the questionnaire, provide a stable base for our conclusions. Furthermore, we were able to confirm *C. difficile* with PCR ribotyping in two thirds of the cases with a positive toxin test, which enabled us to compare types circulating in general practice with those causing disease in hospitals. Similar types were seen in general practice and hospitals in the Netherlands during the study period[27, 28]. As recent evidence suggests that direct transmission of *C. difficile* between hospitalized patients is not the prime route of transmission[29], the large overlap of PCR ribotypes in both settings strengthens the hypothesis of movement of *C. difficile* between both settings.

Our study has limitations. Firstly, we restricted our study to samples sent to a laboratory. Our conclusions are therefore not necessarily generalizable to settings with a different testing incentive. Although Dutch GPs request laboratory diagnostics in 10% to 20% of the gastroenteritis consultations[30] and 20-30% of the GPs in the UK request testing[8, 31], testing incentives in other countries could differ. Secondly, testing strategies in our study include the 'reference standard' and an enzyme immunoassay (EIA), which has a limited negative and positive predictive value in the community[32]. Missing cases due to a false negative toxin test could have resulted in an underestimation of the incidence of CDI. However, the incidence according to the reference standard (used in Tilburg) was only slightly higher. The large sampling fraction in the case control study makes it unlikely that false negative patients were included as controls. However, false positive cases might have occurred. In the majority of the CDI cases (n=130, 86%) no pathogens other than *C. difficile* were found. Additionally, in 13 of the 22 CDI cases with a co-pathogen, the presence of *C. difficile* was confirmed by PCR ribotyping. Therefore, we assume bias according to false positive cases is limited. Thirdly, we would like to stress that the results of Table 5 are dependent on the test that was used. In a setting where different tests for *C. difficile* are used, sensitivity and specificity and therefore the measured incidence of CDI (and the weighted case control analyses of Table 5) can differ. Nonetheless, our conclusion regarding present insufficient testing and suggestions for the future testing are strong and will, in our opinion, hold in a setting with a different test.

Clinical relevance

Although several limitations, our study illustrates that CDI should be included in the differential diagnosis of infectious diarrhoea in general practice, even when the patient was not recently using antibiotics, is young and has no comorbidity. Additionally, it highlights that current Dutch testing strategies are insufficient. Our prediction score greatly improves the detection of CDI, without requiring to test the majority of the CDI patients. However, this prediction score needs validation in a different cohort to ensure it's good performance and cost-effectiveness studies should be done. For now, we recommend to follow current Dutch guidelines or the current UK advice in the Netherlands, which outperforms current practice without testing a large number of samples.

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Part II

Course and outcome of *Clostridium difficile* infections

Chapter 7

All-cause and disease specific mortality in hospitalized patients with *Clostridium difficile* infections; a multicenter cohort study

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Clin Infect Dis 2013

Abstract

Background: Mortality among patients with *Clostridium difficile* infections (CDI) is high. Because of high age and multiple underlying diseases, CDI-related mortality is difficult to estimate. We estimated (CDI-related) mortality in an endemic situation, not influenced by outbreaks and consequently certain patients and *C. difficile* strains.

Methods: Between 2006 and 2009, 13 Dutch hospitals included all hospitalized CDI patients. Nine hospitals individually matched each CDI patient to two control patients, based on ward and time of CDI hospitalization. Survival status was obtained via the Dutch Civil Registration System. Kaplan Meier and Cox-regression were used for survival analysis.

Results: We identified 1,366 patients with CDI (1.33 per 1,000 admissions). All cause mortality risk was 13% after 30 days and 37% after 1 year. The highest mortality was seen among elderly patients and patients with PCR ribotype 027. 317 CDI patients were matched to 317 patients without diarrhea and 232 patients with diarrhea, with a 30-day mortality risk of 5.4% and 8.6% respectively. CDI patients had a 2.5 fold increased 30-day mortality rate compared to controls without diarrhea (Hazard ratio 2.5, 95% CI 1.4-4.3) when adjusted for age, sex and underlying diseases. CDI-related death occurred mainly within 30 days after diagnosis.

Conclusions: Mortality among CDI patients is high, even in an endemic situation. Our study shows that CDI leads to a 2.5 fold increase in 30-day mortality. This highlights the considerable disease burden and clinical impact of CDI, even in absence of an outbreak.

Introduction

Clostridium difficile infections (CDI) emerged in the beginning of the 21st century and are now the leading cause of antibiotic associated diarrhea[1, 2]. Outbreaks in the western world coincided with the emergence of a new type of *C. difficile*: PCR-ribotype 027[3]. This type was postulated to produce more toxin A and B in vitro, the major virulence factors of *C. difficile*[4, 5], and was frequently associated with severe disease in patients[3, 6, 7]. Within this new era of CDI, numerous studies focused on mortality rates among CDI patients. Studies were mainly conducted in outbreak situations or specific populations such as patients treated in Intensive Care Units or Surgical wards[3, 8, 9]. Mortality rates during outbreaks varied, due to the study population and the PCR-ribotype that was associated with the outbreak[8, 10, 11]. Studies in non-outbreak situations are less common. A Canadian surveillance study that identified 1430 CDI patients, of whom 282 were diagnosed during an outbreak, showed that all cause mortality in a setting of low incidence differed considerably from an outbreak situation (15% vs. 23% after 30 days)[12].

Similar to the all cause mortality, CDI-related death increased at least fourfold between 1999 and 2006[13, 14]. However, CDI-related death is difficult to objectify, because the existence of comorbidities is a risk factor for acquisition of the disease. Multiple outbreak investigations have concluded that CDI-related mortality frequently (14-19%) occurs within 30 days[3, 11, 15]. Surprisingly, an endemic study that matched cases and controls on the propensity to develop CDI, concluded that CDI had no direct effect on mortality in the first 60 days. After 3 months, however, the attributable mortality was 6%[16].

We performed a large multicenter cohort study in an endemic situation to estimate the mortality among CDI patients that is not influenced by outbreaks at certain wards or hospitals and consequently certain patient groups. Furthermore, we estimated the CDI-related mortality.

Methods

Study aims

The first aim of our study was to determine the absolute all cause mortality risk of CDI patients. The second aim was to determine CDI-related mortality (1) as the excess mortality when compared to 2 control groups and (2) according to the National Registration of Death certificates.

Patients and definitions

Between July 1st 2006 and April 30th 2009, 13 Dutch hospitals prospectively included CDI patients in the study. The total monthly number of admissions and patient days and type of hospital (university or local) were collected to study the incidence of CDI.

All unformed stool samples of patients who were hospitalized for ≥2 days were tested for *C. difficile* in addition to the patients for whom a *C. difficile* test was requested. The method to detect *C. difficile* toxins differed per hospital; 6 hospitals used the Immunocard (Meridian, bioMérieux), 4 used a cytotoxicity assay and 3 used the Vidas toxin A and/or B enzyme immunoassay (Meridian, bioMérieux). Hospitalized patients with unformed stool and a positive assay for *C. difficile* toxin were considered to have CDI. Patients were included only once.

To calculate the relative mortality rate CDI patients were individually matched to two hospitalized control patients: (1) without diarrhea, (2) with diarrhea and a negative test for the toxin of *C. difficile*. To maximize the feasibility of the study for the participating hospitals, matching was only requested during a pre-specified period of ≥6 consecutive months. Nine hospitals agreed to these terms, consisting of both academic and local hospitals (n=5 vs n=4) (Figure 1). Matching was based on ward of diagnosis and time of hospitalization (control patients were hospitalized within 14 days of the day on which CDI was diagnosed in the CDI patient). When several potential control patients were eligible, the first patient on the alphabetical ward list was chosen. A patient with non-CDI diarrhea fulfilling the matching criteria was not always available.

Demographic data and clinical information such as date of onset of diarrhea, prior underlying diseases, prior medication and prior abdominal surgery were collected for all patients. 'Prior' was defined as: within three months before the start of diarrhea. When symptom onset was unclear, the date of diagnosis was used as a proxy. For non-diarrheal patients 'prior' was defined as: within three months before the reference date. This date was calculated by adding the duration of hospitalization of the matched CDI patient (admission date to start of diarrhea) to the admission date of the patient without diarrhea. Matched CDI patients and controls without diarrhea therefore had a similar duration of hospitalization. In the matched cohort we additionally gathered data on prior admissions and the Charlson Comorbidity Index (CCI) before the current hospitalization[17]. Data were extracted by manual review of the electronic and paper patient chart and contact with the physician in charge. In each facility, data were collected on a standardized

questionnaire by trained research personnel. In patients with CDI, we requested the *C. difficile* strain for PCR-ribotyping[18]. Four hospitals responded well to this request (strains submitted in >75%), while five submitted 36-68% and four hospitals submitted <10%. The study protocol was approved by the Medical Review Ethics Committee of each participating hospital.

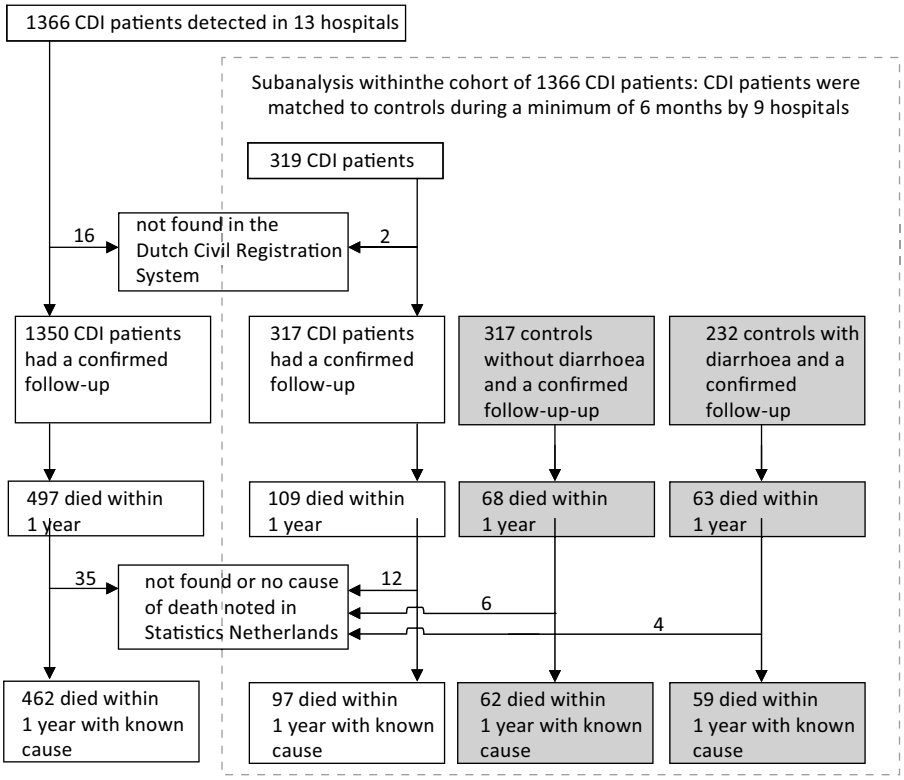

Figure 1. Study populations for analysis of CDI patients and CDI-related deaths.

Outcome measures

Follow-up started at diagnosis or the reference date. Dates of death were ascertained by searching the Dutch Civil Registration System in which dates of death or emigration of all Dutch residents are registered. Information on the cause of death was retrieved from the National Registry of Death certificates, where up to four different causes of death are registered per patient using the International Classification of Diseases, tenth revision (ICD-10)[19]. Patient data was linked to the registry of death certificates by the Netherlands Office of Statistics, thereby guaranteeing anonymity.

The cause of death was known for >90% of the patients that died within one year. We used the following ICD-10 codes for classification of CDI-related mortality:

A04.7 (*Clostridium difficile* enterocolitis); A04.8 and A48.8 (used in the Netherlands to indicate colitis due to *Clostridium* and *Clostridium* infection, not specified as *C. difficile*); the codes of a gastroenteritis of presumed infectious origin, septicaemia due to anaerobes and other bacterial infections of an unspecified site (A09, A41.4 and A49.8). These latter codes in combination with the mention of *C. difficile* in the text of the death certificate are used in England and Wales to select patients with CDI as a cause of death[20]. In the Netherlands the text of death certificates is not available, which might have introduced misclassification.

Statistical analysis

Proportions were compared using the chi-square test. Kaplan Meier was used to calculate the mortality risk and rate and show the 1 year mortality. Proportional hazards modeling (Cox regression) was used to adjust for the effects of age, sex and CCI. To limit confounding by underlying diseases, we additionally adjusted for six ICD-10 Chapters (Method 1), and for medication, admission and abdominal surgery in the three months prior to the onset of diarrhea and admission to an Intensive Care Unit (Method 2). Results are presented as hazard ratios (HR) with accompanying 95% confidence interval (95% CI). Statistical significance was considered to have been reached if a 2-sided p-value was ≤0.05. We used PASW Statistics version 18.0 (SPSS Inc., Chicago) and STATA software package 10.1 (StataCorp, College Station) for our analyses.

Results

Incidence

In the 34-months study period 1,030,202 hospital admissions and 1,366 patients with CDI occurred. The mean incidence was 1.33 per 1,000 admissions (2.1 per 10,000 patient days), varying between 0.74 and 2.30 per 1,000 admissions among the 13 participating hospitals. The monthly variation of CDI incidence within hospitals was small; however, in two hospitals the incidence exceeded 6.00 per 1,000 admissions during one month. No seasonal variation was observed (data not shown).

Table 1. Characteristics of CDI patients and matched controls.

Characteristics	CDI patients (n=1366)		Matched CDI patients (n=317)		Controls without diarrhoea (n=317)		Controls with diarrhoea (n=232)	
Mean age, yr (±SD)	62.6	(±21.6)	61.9	(±21.1)	59.6	(±21.2)	58.4	(±21.6)
Age > 65 years	778 / 1366	57.0%	175 / 317	55.2%	151 / 317	47.6%	100 / 232	43.1%
Male sex, no. (%)	692 / 1366	50.7%	168 / 317	53.0%	165 / 317	52.1%	111 / 232	47.8%
Hospital service, no. (%)								
Internal medicine	777 / 1242	62.6%	196 / 317	61.8%	192 / 317	60.6%	159 / 232	68.5%
Surgery	255 / 1242	20.5%	70 / 317	22.1%	76 / 317	24.0%	44 / 232	19.0%
Health-care association, no. (%)	919 / 1069	86.0%	261 / 303	86.1%	-	-	-	-
Underlying diseases, no. (%)								
Neoplasms	307 / 1113	27.6%	95 / 314	30.3%	91 / 316	28.8%	71 / 232	30.6%
Respiratory system diseases	335 / 1117	30.0%	79 / 315	25.1%	64 / 316	20.3%	41 / 232	17.7%
Digestive system diseases	336 / 1105	30.4%	95 / 316	30.1%	66 / 316	20.9%	81 / 232	34.9%
Circulatory system diseases	488 / 1111	43.9%	172 / 316	54.4%	160 / 316	50.6%	115 / 232	49.6%
Genitourinary system diseases	362 / 1105	32.8%	111 / 316	35.1%	78 / 315	24.8%	59 / 232	25.4%
Endocrine diseases	245 / 1104	22.2%	79 / 316	25.0%	83 / 316	26.3%	63 / 232	27.2%
Antibiotic therapy, no. (%)	953 / 1157	82.4%	264 / 316	83.5%	184 / 314	58.6%	138 / 228	60.5%
Cytostatic agents, no. (%)	165 / 1063	15.5%	51 / 315	16.2%	35 / 316	11.1%	34 / 232	14.7%
Immunosuppressive agents, no. (%)	374 / 1055	35.5%	136 / 312	43.6%	104 / 314	33.1%	90 / 230	39.1%
Abdominal surgery, no. (%)	227 / 1118	20.3%	83 / 314	26.4%	47 / 304	15.5%	46 / 229	20.1%
Admission, no. (%)	-	-	167 / 308	54.2%	93 / 307	30.3%	77 / 229	33.6%
ICU admission, no. (%)	-	-	73 / 313	23.3%	34 / 315	10.8%	36 / 232	15.5%
Most common PCR ribotypes, no. (%)								
014	112 / 689	16.3%	32 / 172	18.6%	-	-	-	-
078	76 / 689	11.0%	21 / 172	12.2%	-	-	-	-
001	57 / 689	8.3%	8 / 172	4.7%	-	-	-	-
027	55 / 689	8.0%	13 / 172	7.6%	-	-	-	-
Charlson Comorbidity Index, no. (%)								
0	-	-	51 / 315	16.2%	65 / 317	20.5%	47 / 232	20.3%
1-2	-	-	118 / 315	37.5%	133 / 317	42.0%	88 / 232	37.9%
3-4	-	-	95 / 315	30.2%	79 / 317	24.9%	60 / 232	25.9%
5+	-	-	51 / 315	16.2%	40 / 317	12.6%	37 / 232	15.9%

Medication use and abdominal surgery were positive when the patient used/experienced this in the 3 months prior to the start of diarrhea. Admission was positive when the patient was admitted to a healthcare facility in the 3 months prior to the start of diarrhea, excluding the current hospitalization. ICU admission (admission to the intensive care unit) was positive when a patient was admitted to an ICU in the 3 months prior to the start of diarrhea or reference date (could be during the current hospitalization).

CDI cohort

The mean age of the 1,366 CDI patients was 63 years, half of them were male (50.7%). Eighty-six percent had healthcare-associated CDI (development of diarrhea >48 hours after admission or <12 weeks after discharge). Underlying diseases were common and 82.4% received antibiotic therapy in the three months prior to diarrhea (Table 1). The most frequently found PCR-ribotype among CDI patients was type 014 (112/689; 16.3%). Other frequently found types were 078 (11.0%), 001 (8.3%) and 027 (8.0%). Patients with a typing result resembled those without, with respect to age (mean 62.4 vs 62.8), underlying diseases (mean CCI 2.6 vs 2.8), medication use and outcome.

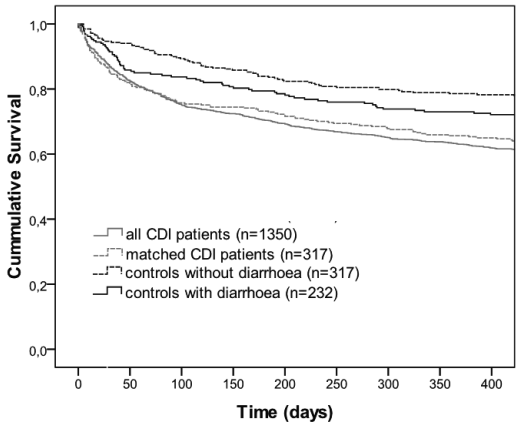

Figure 2. Mortality rate of all CDI patients and the matched cohort (CDI patients and matched control patients) during the first year of inclusion.

	< 30 days		< 3 months		< 6 months		< 1 year	
Death, no. (%)								
all CDI patients	177/1350	13.1%	319/1350	23.6%	401/1350	29.7%	497/1350	36.8%
matched CDI patients	47/317	14.8%	74/317	23.3%	85/317	26.8%	109/317	34.4%
controls without diarrhoea	17/317	5.4%	31/317	9.8%	51/317	16.1%	68/317	21.5%
controls with diarrhoea	20/232	8.6%	38/232	16.4%	48/232	20.7%	63/232	27.2%

Outcome Of 1350 CDI patients with known follow-up, 177 patients died within 30 days, accounting for a mortality risk of 13.1% (47.3 per 10,000 person years). One year after diagnosis 497 patients had died (36.8%) (Figure 2) and in 1% (10/1145) a colectomy was performed. The 30-day mortality increased with age (Table 3), with the highest case fatality observed for persons between 80 and 89 (52/244; 21.3%) and above 90 years (8/39; 20.5%). PCR-ribotype 027 was associated with the highest

30-day mortality (12/55; 21.8%). Compared to patients with CDI due to other PCR-ribotypes, patients with type 027 had a significantly higher mortality risk (21.8% vs 11.3%; Cox regression analysis: p=0.02; HR 2.1, 95% CI 1.1-3.8). The mortality among patients with type 027 remained significantly higher after adjustment for age and sex (p=0.04; HR 1.9, 95% CI 1.0-3.5).

Table 2. Mortality risk stratified by PCR-ribotype and age.

		Deaths		
	Total *n=1350*	**<30 days** *n=177*	**<3 months** *n=319*	**<1 year** *n=497*
Age (decades)				
≤9	58	0,0%	1,7%	6,9%
10-19	40	2,5%	7,5%	15,0%
20-29	33	6,1%	9,1%	12,1%
30-39	52	1,9%	3,8%	15,4%
40-49	90	10,0%	14,4%	28,9%
50-59	191	12,0%	18,8%	28,3%
60-69	252	11,9%	23,0%	34,5%
70-79	351	14,5%	29,9%	45,6%
80-89	244	21,3%	34,8%	51,2%
≥90	39	20,5%	33,3%	59,0%
PCR ribotype				
014	111	10,8%	20,7%	32,4%
078	76	14,5%	23,7%	38,2%
001	57	15,8%	22,8%	33,3%
027	55	21,8%	32,7%	40,0%
other	387	10,1%	20,2%	34,9%
no type result	664	14,2%	25,5%	38,6%

Mortality risk stratified by age decades. Additionally, stratification displayed the four most frequently found PCR ribotypes, a group of all other types combined (n=387) and a group of patients with CDI but without a PCR ribotype result.

Matched cohort

The 317 CDI patients that were matched to controls without diarrhea (n=317) and, if available, controls with diarrhea (n=232) resembled the total cohort of CDI patients (Table 1). Statistically significant differences between matched CDI patients and the total population were: lower frequency of respiratory diseases (25% vs 30%), higher frequency of circulatory diseases (54% vs 44%) and more frequent use of immunosuppressive agents (43% vs 36%). Treatment for CDI consisted of metronidazole (234/309; 76%), vancomycin (2%) or a combination of both (11%). Eleven percent was not treated for CDI.

The mean age of controls without and with diarrhea was 60 and 58 years, respectively, compared to 62 years in matched CDI patients (p=0.17 and p=0.03). Underlying diseases were more prevalent in CDI patients, except for endocrine diseases. The mean CCI was higher among CDI patients than among controls patients without and with diarrhea: 2.68, 2.28 and 2.42, respectively (p=0.01 and p=0.04). Sixteen percent of CDI patients (51/317) had an index of five or above.

Outcome Mortality of CDI patients and controls is displayed in Figure 2 and Table 3. Among matched CDI patients, 14.8% died within 30 days (53.9 per 10,000 person years), which was similar to the cohort of CDI patients (percentage: p=0.21). The 30-day mortality among control patients without and with diarrhea was considerably lower: 5.4% and 8.6% (p<0.01 and p=0.01), respectively.

Within the first 30 days, mortality among CDI patients was 2.9 times higher than among non-diarrheal controls (HR 2.9, 95% CI 1.7-5.1). After adjustment for baseline differences in age, sex and CCI, CDI was still associated with a 2.5 fold increased 30-days mortality rate (95% CI 1.4-4.3). The hazard ratio decreased to 1.8 (95% CI 0.9-3.5) and 0.9 (95% CI 0.6-1.4) within 3 months and one year, respectively. Overall, CDI was associated with a 1.5 times increased mortality (95% CI 1.1-2.0) in the first year. Results were similar when additional adjustments for underlying diseases or medication and admissions were made (Method 2 and 3 in Table 3), or when the in-hospital mortality was assessed in stead of the 30-day mortality (HR according to Method 1: 2.3, 95% CI 1.1-4.7). Post-discharge mortality (a proxy for long-term mortality) with up to one year follow-up was not significantly different in matched CDI patients and non-diarrheal controls (HR according to Method 1: 1.0, 95% CI 0.7-1.5).

When CDI patients were compared to controls with diarrhea, the hazard ratio for 30-day mortality was 1.9, 95% CI 1.1-3.3 (58.0 vs 29.9 per 10,000 patient years). In multivariable analysis, this mortality rate was 1.6 (95% CI 0.9-2.8).

PCR-ribotypes were known for 25 patients with CDI-related codes on their death certificates. Type 078 (6/25; 24%), 045 and 001 (both 3/25; 12%) were the most common. The primary cause of death was a neoplasm or a disease of the respiratory or circulatory tract in most CDI patients as well as controls (Table 4).

Table 3. Absolute and relative mortality rates in the matched cohort.

	Absolute mortality rate				Rate Ratio, HR (95% CI)			
	deaths / py at risk	*deaths per 10,000 py*	*deaths / py at risk*	*deaths per 10,000 py*	*Unadjusted*	**Method 1** *Adjusted for age, sex and Charlson Index*	**Method 2** *Method 1 + underlying diseases (ICD-10)*	**Method 3** *Method 1 + medication + admission*
	Matched CDI patients		**Controls without diarrhea**					
< 30 days	47 / 8717	53.9	17 / 9211	18.5	2.9 (1.7-5.1)	2.5 (1.4-4.3)	2.4 (1.3-4.2)	2.6 (1.4-4.9)
30 days - 3 months	27 / 15337	17.6	14 / 17609	8.0	2.2 (1.2-4.2)	1.8 (0.9-3.5)	1.9 (1.0-3.7)	2.1 (1.0-4.4)
3 months - 1 year	35 / 61758	5.7	37 / 72016	5.1	1.1 (0.7-1.7)	0.9 (0.6-1.4)	0.9 (0.6-1.5)	1.0 (0.6-1.6)
Overall	109 / 85812	12.7	68 / 98836	6.9	1.8 (1.3-2.4)	1.5 (1.1-2.0)	1.5 (1.1-2.0)	1.6 (1.1-2.3)
	Matched CDI patients		**Controls with diarrhea**					
< 30 days	37 / 6379	58.0	20 / 6681	29.9	1.9 (1.1-3.3)	1.6 (0.9-2.8)	1.6 (0.9-2.7)	1.5 (0.9-2.7)
30 days - 3 months	19 / 11121	17.1	18 / 12013	15.0	1.1 (0.6-2.2)	0.9 (0.5-1.8)	0.9 (0.5-1.8)	0.9 (0.4-1.7)
3 months - 1 year	25 / 44577	5.6	25 / 49650	5.0	1.1 (0.6-1.9)	1.0 (0.5-1.7)	1.0 (0.5-1.7)	0.9 (0.5-1.6)
Overall	81 / 62077	13.0	63 / 68344	9.2	1.4 (1.0-1.9)	1.2 (0.8-1.6)	1.2 (0.8-1.6)	1.1 (0.8-1.6)

The Rate Ratio was calculated using Cox regression analysis.
HR: Hazard ratio.
py: patient years.

In Method 1 we used age (continuous variable), sex and Charlson Comorbidity index (continuous variable) as adjusting variables. Method 2 adjusted for these 3 variables, supplemented with adjustment for the six most frequently found ICD-10 Chapters among CDI patients and controls (dichotomous variables: Chapter II Neoplasms; Chapter IV Endocrine, nutritional and metabolic diseases; Chapter IX Diseases of the circulatory system; Chapter X Diseases of the respiratory system; Chapter XI Diseases of the digestive system; Chapter XIV Diseases of the genitourinary system). In Method 3 we adjusted for age, sex, Charlson Comorbidity index, medication in the previous 3 months (antibiotics, immunosuppressives, chemotherapeutic agents), prior admission, prior abdominal surgery and admission to an Intensive Care Unit in the 3 months prior to diarrhea (see Table 1 for exact information on the occurrence of these variables in CDI patients and controls).

CDI-related mortality according to death certificates

Of the 497 patients that died within 1 year, death certificates could be accessed in 93% (462/497). According these certificates (Table 4), the cause of death was related to CDI in 46 patients (10.0% of all deaths; 3.7% of all CDI patients). Three certificates specifically coded death due to *C. difficile* enterocolitis, 36 had a code for *Clostridium* infection/colitis and 7 had one of the unspecific codes possibly related to CDI (2x A09; 5x A41.4). Most (72%; 33/46) CDI-related deaths occurred within 30 days. Eleven other patients died within 3 months, only two patients died thereafter (105 and 193 days, respectively). In contrast, no control patients had a primary or secondary cause of death related to CDI.

Table 4. Primary cause of death according to death certificate data of CDI and control patients that died within one year.

	CDI patients (n=1350)		**Controls without diarrhea (n=317)**		**Controls with diarrhea (n=232)**	
	n	*%*	*n*	*%*	*n*	*%*
Death within one year	497		68		63	
Known cause of death	462	93.0	62	91.2	59	93.7
Primary cause of death						
Infectious and parasitic diseases	36	7.8	0	0.0	3	5.1
Neoplasms	170	36.8	27	43.5	28	47.5
Digestive organs	38	8.2	10	16.1	8	13.6
Lung / bronchus	26	5.6	2	3.2	1	1.7
Lymphoid / haematopoietic tissue	48	10.4	8	12.9	12	20.3
Endocrine, nutritional and metabolic diseases	14	3.0	5	8.1	0	0.0
Circulatory system	88	19.0	16	25.8	8	13.6
Ischaemic heart disease	18	3.9	4	6.5	1	1.7
Respiratory system	54	11.7	5	8.1	9	15.3
Digestive system	37	8.0	6	9.7	4	6.8
Genitourinary system	25	5.4	1	1.6	2	3.4
Other	38	8.2	2	3.2	5	8.5
C. difficile-related death (primary or secondary cause)	46	10.0 #	0	0.0	0	0.0

Causes of death that were noted as primary cause of death. Causes of death are listed by ICD-10 Chapter and then by ICD-10 Block: e.g. Chapter II is referred to as "Neoplasms", Block C15-C26 is referred to as "digestive organs" (nomenclature as displayed in the ICD-10) (19). *C. difficile*-related death was determined by selecting those patients who had ICD-10 code A04.7, A04.8, A48.8, A09, A41.4 or A49.8 as a cause of death. Up to four different causes of death are registered.
10% of all deaths is equal to 3.7% of all CDI patients.

Discussion

During the study period, we experienced a stable, low incidence of CDI in hospitals. Even in this endemic setting mortality among hospitalized CDI patients was high: 13% within 30 days. This percentage was only slightly lower than observed during outbreaks (15% to 25% within 30 days)[3, 8, 11, 12]. Compared to matched patients without diarrhea, the 30-day mortality rate of CDI patients in our study was increased2.5 fold; a rate estimate consistent with a Canadian study with a ten-fold higher incidence[21]. This highlights CDI as a serious healthcare problem, even in absence of an outbreak.

Our study showed that CDI-related mortality occurred mainly within 30 days; long-term consequences of CDI (mortality after 90 days) were small. As the one-year mortality in CDI patients was 50% times higher (adjusted Hazard ratio) than in controls (1-year mortality risk: 21.5%), we estimate that CDI-related mortality risk is about 10% (50% of 21.5%). Given a yearly incidence of 2700 CDI patients in the Netherlands[22], about 270 deaths annually (10%) are estimated to occur as a consequence of the infection. This corresponds to 0.2% of all deaths in the Netherlands. Although this number is lower than the number of CDI related deaths in England and Wales (1.1% of all deaths; derived from death certificates)[20], it underscores the importance of CDI as a cause of death in the Netherlands.

According to the death certificates, 3.7% of the CDI patients died as a consequence of CDI. This is clearly less than our estimated CDI-related mortality risk (10%). Although we used relatively non-specific ICD-10 codes, in addition to a specific code (A04.7), to estimate CDI-related death, a majority (72%) of the patients with CDI-related ICD-10 codes died within 30 days of their diagnosis. Furthermore, non-specific ICD-10 codes were not observed in control patients. Therefore, we believe that we did not overestimate CDI-related mortality. Rather, our study indicates that death certificates lack sensitivity to provide a correct estimate of the CDI-related mortality, which is in accordance with studies from the UK[25] and Canada[26], who report that death certificates may be inaccurate to investigate CDI-related death[14, 27].

A large number of *C. difficile* strains were available for further typing (n=689; 50.4%) and we were able to relate these types to patient characteristics. Since patients without typing results resembled typed patients with respect to clinical characteristics and outcome, and most hospitals either responded well or did not respond at all to the typing request, we believe selection bias based on severity of disease is limited. The PCR-ribotypes found in our study are also common in Europe[28]. The finding that type 027 was significantly associated with a higher 30-

day mortality rate, adds evidence to the hypothesis that type 027 has hypervirulent characteristics[3, 11, 29].

The multicenter approach and large timeframe of our study resulted in 1,366 CDI patients during more than a million hospital admissions. This design enabled us to analyze CDI in a low incidence environment with numerous different PCR-ribotypes, which ensured us that our conclusions were not substantially influenced by outbreaks with specific types among specific groups of patients. Another strength of our study is that data were complete and carefully obtained since they were extracted by manual review of patient charts and contact with the treating physician after which outcome data was checked using national registries.

Our study has few limitations. First, we had access to two control groups that were selected by the criterion of presence vs absence of diarrhea. A control group without considering this criterion would have been more representative[31, 32]. Because only ten percent of the hospitalized patients experience diarrhea during their hospitalization, the comparison of CDI patients to controls without diarrhea was considered the most reliable. However, when we analyzed both control groups in one group, we found a similar one-year CDI-related mortality, which therefore did not influence our conclusions. A second limitation is the possibly that we failed to identify all CDI patients due to the low sensitivity of enzyme immunoassays (EIA)[33]. Including EIA-negative patients as diarrheal controls could have led to under-estimation of the CDI-related mortality rate, which is a second motive to report the comparison of CDI patients to controls without diarrhea as the most reliable. Finally, as with any observational study we cannot rule out residual confounding due to underlying diseases in the estimation of the CDI-related mortality. In our analysis we adjusted for age, sex and underlying diseases (using three methods). Matching accounted for hospital and ward of admission. Additionally, CDI patients and controls without diarrhea had a similar duration of hospitalization. By taking into account parameters for chronic underlying diseases (e.g. Charlson Comorbidity index) and acute disease (duration of hospitalization), we think we provided a good estimate of the true excess mortality in CDI patients.

In conclusion, our large multicenter study shows that all cause mortality rates among CDI patients are high and that CDI increases mortality 2.5 fold, even in an endemic situation. This highlights the considerable disease burden and clinical impact of CDI, even in absence of an outbreak and emphasizes the need for preventive strategies and novel therapeutic approaches.

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Chapter 8

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

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Clin Infect Dis 2012

Abstract

Non-severe and severe *Clostridium difficile* infection (CDI), which carries a higher risk 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, consisting of 1105 patients. 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 × 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 (using 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 χ^2 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% (13/1102) of patients 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, 0.68–1.02; Figure 1). Neither fever nor leukocytosis was associated with a lower probability of resolution of diarrhea (HR, 1.08 [95% CI, 0.61–1.91] and HR, 1.02 [95% CI, 0.84–1.24], respectively). 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 × 10^9/L	38/153	2.29	1.63–3.21
	≤15 × 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.
Creatinine conversion: 1 µmol/L is equal to 0.0113 mg/dl. Therefore: 133 µmol/L is equal to 1.50 mg/dl.

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).

Table 2. Determinants of Recurrence.

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

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

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.
Creatinine conversion: 1 µmol/L is equal to 0.0113 mg/dl. Therefore: 133 µmol/L is equal to 1.50 mg/dl.

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 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 × 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.

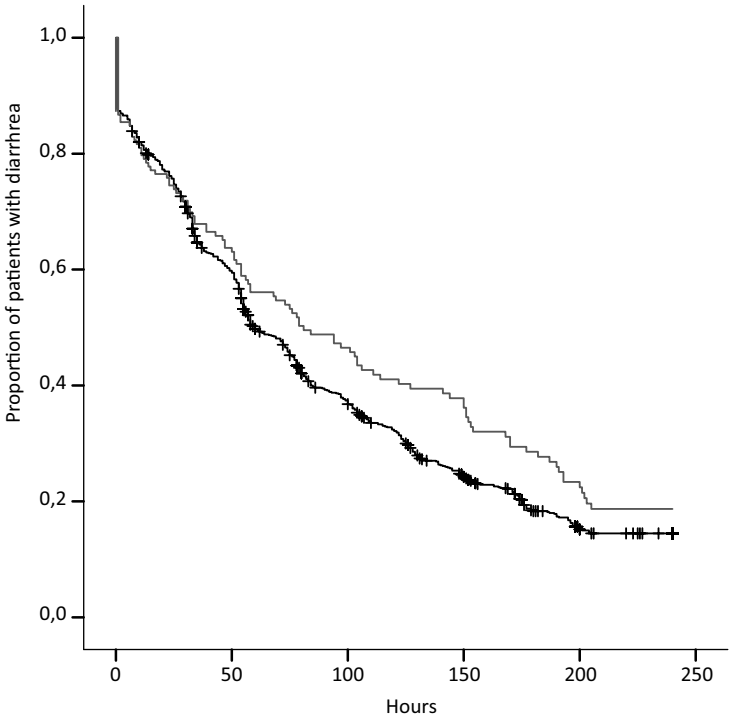

Figure 1. Kaplan-Meier analysis of time to resolution of diarrhea for patients with and without renal failure. Hazard ratio was 0.83 (95% confidence interval: 0.68–1.02).

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.

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Chapter 9

Clostridium difficile infection due to binary toxin positive strains

Response to: "Binary Toxin and Death after *Clostridium difficile* Infection" by Bacci et al.

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Emerg Infect Dis 2013

To the Editor: With interest, we read the article of Bacci et al. in which they conclude that *Clostridium difficile* strains containing the binary toxin gene were associated with a higher case fatality after 30 days, even when the analysis was stratified for PCR-ribotype[1]. Although an appealing conclusion, in our opinion the study was severely limited by selection bias and confounding by underlying diseases. First, in Danish patients with a *Clostridium difficile* infection (CDI) isolates were characterized only when they were isolated during outbreaks or from patients with severe CDI or if they were found to be moxifloxacin resistant. Therefore, selection bias was likely to occur. Second, adjustment for concurrent conditions was not performed. This was especially warranted because outbreaks on specific hospital wards (e.g., intensive care units) could have influenced the all-cause mortality rate. Last, the selection of specific patients and strains questions the generalizability of the authors' conclusion.

In an approach to confirm the findings of Bacci et al.[1], we used data from a cohort study conducted during 2006 – 2009 in 13 Dutch hospitals[2]. A total of 1,350 consecutive hospitalized patients with unformed feces and a positive *C. difficile* toxin test result were included in the study. We checked the 30-day survival for study patients in the Dutch Civil Registration System. For 626 (46%) of the patients, a *C. difficile* strain was available for PCR ribotyping and binary toxin gene characterization. Patient data (e.g., age, sex, hospitalization, and antibiotic use in the 3 months before onset of diarrhea) were collected by review of the electronic and paper patient chart and by contacting the treating physician. Underlying diseases present at hospital admission were classified into seven disease categories (Table footnote). In addition, during at least six months, the Charlson comorbidity index at admission was determined in nine of the 13 hospitals (total of 357 CDI patients). Proportional hazards modeling was used for survival analysis. The Medical Review Ethics Committee of the Leiden University Medical Center approved this study.

During the study period, CDI was endemic in all hospitals in the cohort study (13 cases per 10,000 admissions). The all cause risk for dying within 30 days was 22% (12/55) for persons infected with binary toxin positive-027 strains, 15% (15/100) for those infected with binary toxin positive-non-027 strains and 11% (50/471) for those infected with binary toxin-negative strains (Table). Selection bias (e.g., by primarily characterizing isolates of patients with severe disease), was unlikely because the number of deaths among CDI patients without strain characterization (100/724 [14%]) was similar to that among patients with a characterized strain (77/626 [12%]; p = 0.41). Thirty-day mortality rates were significantly higher among patients with CDI due to type 027 strains than among patients with binary toxin–negative strains

(hazard ratio [HR] 2.2); additional adjustment for age and concurrent condition(s) resulted in a relatively constant HR of 2.0–2.4. Patients with CDI due to binary toxin–positive non-027 strains did not have a substantially higher 30-day mortality rate (HR 1.5); additional adjustment for age and concurrent condition(s) lowered the HR to 1.1–1.4, depending on the method of adjustment.

Table 1. Crude and adjusted hazard ratios of 30-day mortality rate, stratified by PCR-ribotype.

			Relative 30-day mortality (Hazard Ratio)				
	Absolute 30-day mortality		**No adjustments**	**Adjusted for age**	**Adjusted for age and comorbidity**		
	N	*% (95% CI)*			*Method 1*	*Method 2*	*Method 3*
Binary toxin positive strains							
027 (n=55)	12	22 (13-35)	2.2 (1.2-4.2)	2.0 (1.1-3.8)	2.4 (1.1-5.5)	2.0 (0.8-5.4)	2.0 (0.7-5.5)
Non-027 (n=100)	15	15 (9-23)	1.5 (0.8-2.6)	1.4 (0.8-2.5)	1.3 (0.6-2.7)	1.1 (0.5-2.8)	1.1 (0.4-2.9)
Binary toxin negative strains (n=471) $	50	11 (8-14)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Binary toxin unknown (n=724) #	100	14 (11-17)	1.3 (0.9-1.9)	1.3 (0.9-1.9)	1.4 (0.9-2.2)	1.5 (0.9-2.3)	1.3 (0.7-2.4)

* In the model, age and Charlson index were added as continuous variables; all others were dichotomous. HR, hazard ratio. Method 1, adjusted for age and history of admissions and antimicrobial drug use in the prior 3 months. Method 2: adjusted for age; diseases of the respiratory, digestive, circulatory, and genitourinary systems;
endocrine diseases; neoplasms and other diseases; history of admissions and antimicrobial drug use in the prior 3 months. Method 3, adjusted for age; history of admissions; antimicrobial drug use in the prior 3 months and Charlson comorbidity index.
$ Binary toxin–positive non-027 strains belonged to 8 different PCR ribotypes (76% type 078).
# Binary toxin–negative strains belonged to 64 different PCR ribotypes (23% type 014).Method 1: correction for age and history of admissions and history of antibiotic use (3 months).

In accordance with findings in the Danish study, we observed a high 30-day mortality rate among persons infected with type 027 isolates. The 30-day mortality rate was lower among persons infected with non-027 binary toxin–positive isolates, especially after correction for concurrent condition(s); however, confidence intervals overlapped with those for type 027. Therefore, we cannot statistically contradict the conclusion of Bacci et al.[1]. Nevertheless, because mortality rates in our study among patients with non-027 type CDI strongly resembled mortality rates among patients with CDI caused by binary toxin–negative isolates and because the Danish study was prone to bias and lacked adjustment for confounding, we think that the results of Bacci et al.[1] should be interpreted with caution. Furthermore, a large clinical

study from 2008 concluded that *C. difficile* type 078, which is the most frequently found binary toxin positive non-027 strain, was not associated with a high all-cause mortality rate[3]. A more recent publication confirmed this finding[4]. Therefore, in our opinion, there is currently no convincing epidemiologic proof that binary toxin is a marker for infection with virulent *C. difficile.*

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Chapter 10

Predicting a complicated course of *Clostridium difficile* infection at the bedside

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Submitted

Abstract

Background: *Clostridium difficile* infections (CDI) are a common cause of antibiotic associated diarrhea and associated with CDI-related mortality in approximately 10%. To date, there is no prediction model in use that guides clinicians to identify patients at high risk for complicated CDI.

Methods: From 2006 to 2009, nine Dutch hospitals included hospitalized CDI patients in a prospective cohort. Potential predictors of a complicated course (ICU admission, colectomy or death due to CDI) were evaluated in uni- and multivariate logistic regression. A score was constructed which was internally validated by bootstrapping. Furthermore, a pilot external validation was performed.

Results: Twelve percent of 395 CDI patients had a complicated course within 30 days after diagnosis. Age (≥85 years OR 4.96; 50-84 years 1.83), admission due to diarrhea (OR 3.27), diagnosis at the ICU department (OR 7.03), recent abdominal surgery (OR 0.23) and hypotension (OR 3.25) were independent predictors of a complicated course. These variables were used to construct a prediction model. A score subsequently classified patients into high risk (39% with a complicated course), intermediate (16%), low (5%) or virtually no risk to experience a complicated course. The score performed well after internal validation (AUC 0.78) and a pilot external validation among 139 patients showed similar good performance (AUC 0.73).

Conclusions: We present an easy-to-use, clinically useful risk score that is capable of categorizing CDI patients according to their outcome. Since classification is available at diagnosis, it could have major implications for e.g. treatment choice.

Introduction

Clostridium difficile infection (CDI) commonly presents as a colitis, which occurs when toxin is produced by the bacterium. Symptoms may include cramps, fever, abdominal pain or signs of an ileus or peritonitis; diarrhea is almost always present. Inflammation of the gut may be so severe that hypotension, perforation or a toxic megacolon occurs[1, 2]. The number of patients that die as a consequence of CDI increased when a virulent *C. difficile* strain, PCR ribotype 027, emerged in 2002. CDI is now found to increase the absolute risk of death within 30 days by approximately 10 percent[3, 4].

Vancomycin and metronidazole are currently the most frequently used drugs to treat CDI, but newer treatment options, such as the recently licensed drug fidaxomicin, are now available[5]. This drug has been shown to be as effective as vancomycin in the treatment of CDI, but the population that benefits most from this new but costly treatment remains to be determined. In patients with severe symptoms of CDI, vancomycin treatment is superior to metronidazole[6, 7]. Because severe symptoms are associated with a complicated course (e.g. death), it is important to identify patients at risk of a complicated course and use this as a guide towards treatment[2, 8]. In an attempt to characterize patients who die due to CDI, several risk factors have been described, including advanced age, concomitant use of antibiotics, fever, admission to the intensive care unit and presence of leucocytosis, elevated creatinine or low serum albumin[7, 9-12]. Furthermore, *C. difficile* specific factors such as PCR ribotype have been associated with mortality due to CDI[9]. In spite of evidence for useful predictors of a complicated course, no clinically useful prediction model has been developed to date[13].

In this study, we aim to define prognostic markers for a complicated course of CDI, using variables that are available at a patient's bedside at time of diagnosis. Next, we aimed to develop an easy-to-use prediction rule that could help physicians to identify patients at risk of a complicated course of CDI.

Patients and methods

Patient selection

From March 2006 to May 2009, nine Dutch hospitals (5 academic, 4 community) prospectively included hospitalized patients with CDI in a cohort study. Hospitals participated for a minimum of six consecutive months in the three year study

period. Patients from all departments and co-morbidities were considered eligible. CDI was defined as the presence of diarrhea (≥3 unformed stools per 24-h period) and a positive *C. difficile* toxin test. In addition to testing on clinical suspicion of the treating physician, all patients with diarrhea who were hospitalized for two or more days were routinely tested for *C. difficile*. The toxin test that was used differed per hospital according to the local standard. Four hospitals used the ImmunoCard Toxins A&B (Meridian), three used a cytotoxicity assay, one used the Premier Toxins A&B (Meridian) and another hospital used the VIDAS *C. difficile* A&B test (bioMerieux). For every patient, only a single inclusion in the study was possible. The study was approved by the Institutional Review Ethics Boards.

Data collection

Patient information was collected by a study physician (AG) and registered on a standardized questionnaire, using patient records, the electronic medical information system and by consulting the physician in charge. Demographic characteristics such as age, sex, hospital and department of diagnosis were collected. Information on risk factors for CDI present in the three months prior to the onset of diarrhea was collected and included previous medication (antibiotics, immunosuppressive agents, chemotherapeutic agents, antacids and proton-pump inhibitors) and hospital admissions. Data concerning underlying medical conditions were classified using the 10th edition of the International Classification of Diseases and the Charlson's Comorbidity Index[14]. At the day of diagnosis (plus or minus one day), signs and symptoms during physical examination were recorded, i.e.: fever (temperature >38.5°C), macroscopic blood in the stool, hypotension (systolic blood pressure below 100 mmHg and/or diastolic blood pressure below 60 mmHg), abdominal pain. Serum creatinine was recorded before the onset of diarrhea.

Variables had missing data in less than 3% of patients, except for fever, hypotension and bloody diarrhea, which were incomplete in 10-13%. Creatinine values were not registered in one hospital (13%). To account for missing data in multivariable analysis, values were imputed using multiple imputation. This method is appropriate when values are missing at random (MAR)[15], which seemed reasonable to assume in our study because variables that were predictive of the missing data were determined. All potential predictors, the outcome variable and nine additional variables were included in the imputation procedure.

Identification of *C. difficile* was done at the LUMC by the detection of the *glu*D gene using a PCR. All positive isolates were subsequently PCR-ribotyped as previously described[16, 17].

Outcome measurement

Thirty days after diagnosis the course of CDI was considered by consensus of the treating physician and a study physician (MH or AG). A complicated course was defined according to international recommendations[18, 19]: (1) death as a direct or indirect consequence of CDI, (2) admission to the intensive care unit due to CDI, (3) colectomy due to CDI. Survival status of all patients was checked using the Dutch Civil Registration System in which all Dutch inhabitants are registered.

Predictors of a complicated course of CDI

Based on previous research we selected potential predictors of a complicated course of CDI that could be obtained at time of diagnosis, including age, department of diagnosis, use of antibiotic agents, Charlson's Comorbidity Index and creatinine count[3, 9-11, 20, 21]. Additionally, we selected sex, hospital of diagnosis (academic or community), location of onset diarrhea (healthcare or community), reason for admission (diarrhea or other), some well known risk factors for acquiring CDI (medication and interventions) and signs and symptoms that were recorded during physical examination as potential predictors, with the exception of abdominal pain, which was deemed too subjective. Potential predictors were analyzed in univariate logistic regression analysis. Multivariable logistic regression was performed for all potential predictors with a p-value <0.50 in univariate analysis. Subsequently, the model was reduced by stepwise excluding variables with a p-value of >0.10 based on the log likelihood ratio test (backward selection). Therefore, the strongest predictors remained in the final model. Results were displayed as Odds ratios (OR).

Prediction rule development, performance and internal validation

Any prognostic model shows too optimistic performance in the dataset from which it is developed (over-fitting)[22]. To adjust for this optimism and to validate the model, we used bootstrapping techniques. During this process, the model is constructed numerous times (n=200) using a subset of the dataset to predict the outcome of the other part of the dataset. This way, the optimism can be quantified with a number (shrinkage factor). The regression coefficients of the final model were multiplied with the shrinkage factor and subsequently rounded to integers to construct a simple

prediction rule. For each patient we calculated a summed score. The discriminative ability of our model was expressed by calculation of the area under the Receiver Operating Characteristic curve (ROC area), which ranges from no discrimination (0.5) to perfect discrimination (1.0). A simplified version of the prognostic rule was constructed, to divide patients into a low, medium and high risk category. Similarly, this simplified rule was tested for its discriminative ability, sensitivity and specificity. Furthermore, performance was assessed by calculating the positive and negative predictive values and diagnostic accuracy.

Sensitivity analyses and pilot external validation

Several sensitivity analyses were performed including (1) restriction to patients ≥15 years, (2) restriction to patients who were treated for CDI with metronidazole and (3) a complete case analysis. A small cohort (n=139) was used as a pilot of external validation. This cohort consisted of all CDI patients diagnosed between May 2009 and May 2011 in a single hospital (Radboud University Medical Center, Nijmegen, the Netherlands). This hospital also participated in the derivation study between 2006 and 2009; definitions of CDI and outcome were equal to those used to construct the prediction rule.

Analyses were done in PASW Statistics version 17.0 (SPSS Inc., Chicago) and R version 2.12.2, package Design and pROC (The R Foundation for Statistical Computing, Vienna).

Results

In total, 395 patients with CDI were included. Their median age was 65 years (IQR 52-77), 55.7% of the population was male. Three months prior to the onset of diarrhea, 85.0% had used antibiotic therapy and 54.7% had been admitted to a healthcare facility. Abdominal pain (54%), fever (60%) and hypotension (30%) were frequently present at time of diagnosis, whereas bloody diarrhea (15%) was present in a minority of the patients. Patient characteristics are displayed in Table 1.

Table 1. Univariate analysis of potential predictors for the development of a complicated course due to CDI.

	CDI patients (N=395)		Severe course due to CDI * yes		no		Odds ratio 95% CI		P-value
	N	*%*	*N*	*%*	*N*	*%*			
Demographic characteristics									
Age									
≤ 49 years	85	22%	6	13%	79	23%	1	reference	0.01
50 - 84 years	275	70%	31	67%	237	70%	1.72	0.69-4.28	
≥ 85 years	35	9%	9	20%	23	7%	5.15	1.66-16.0	
Male sex	220	56%	24	52%	191	56%	0.85	0.46-1.57	0.59
Academic hospital	266	67%	23	50%	239	71%	0.42	0.22-0.78	0.01
Department of diagnosis									
Other departments	293	74%	35	76%	251	74%	1	reference	<0.01
Surgery	83	21%	4	9%	78	23%	0.37	0.13-1.07	
Intensive Care Unit	19	5%	7	15%	10	3%	5.02	1.80-14.0	
Medication and intervention history **									
Cytostatic agents	64	16%	7	15%	55	16%	0.91	0.39-2.15	0.84
Immunosuppressive agents	172	44%	21	47%	146	44%	1.13	0.60-2.10	0.71
Proton pump inhibitors	251	64%	34	76%	211	63%	1.82	0.89-3.71	0.10
Recent abdominal surgery	110	28%	4	9%	105	31%	0.21	0.07-0.59	<0.01
Recent admission	210	55%	28	61%	177	54%	1.37	0.71-2.49	0.38
Antibiotic agents	335	85%	34	74%	293	87%	0.44	0.21-0.90	0.03
Clinical characteristics									
Charlson Index									
0	59	15%	7	15%	52	15%	1	reference	0.53
1 - 2	150	38%	14	30%	134	40%	0.78	0.30-2.03	
3 - 4	120	31%	15	33%	101	30%	1.10	0.42-2.87	
> 5	64	16%	10	22%	50	15%	1.49	0.53-4.21	
Diarrhea as reason for admission	104	27%	23	50%	78	23%	3.31	1.76-6.22	<0.01
Healthcare onset diarrhea	283	72%	28	61%	248	74%	0.55	0.29-1.04	0.06
Fever	208	60%	25	66%	174	59%	1.36	0.67-2.76	0.40
Hypotension	117	30%	25	63%	88	30%	3.86	1.94-7.68	<0.01
Bloody diarrhea (macroscopic)	52	15%	7	16%	44	15%	1.14	0.48-2.71	0.77
Laboratory parameter									
Creatinine count prior to start of diarrhea									
<90	199	58%	17	43%	178	61%	1	reference	0.05
>90	109	32%	16	40%	89	30%	1.88	0.91-3.90	
Dialysis	33	10%	7	18%	25	9%	2.93	1.11-7.77	

* Outcome is missing for 10 patients (2.5%), therefore the maximum number of patients is 46 with a severe course and 339 without a severe course.

** Medication and intervention history was gathered from the three months prior to the start of diarrhea.

Within the first 30 days after diagnosis, 88.2% of the patients received antibiotic treatment for CDI. Most frequently, metronidazole was used (74.3%). A combination of metronidazole and vancomycin was used in 11.3% and vancomycin monotherapy in 2.6%. Sixty-five patients (16.5%) died within 30 days after the diagnosis, of which 38 (9.9%) were related to CDI. Five patients had a colectomy and three were admitted to the intensive care unit due to CDI, therefore, a complicated course due to CDI was observed in 46 patients (11.9%).

The PCR ribotype causing CDI was known for 206 patients (52.2%); the most frequently found types were 014 (16.9%), 078 (12.1%), 001 (8.7%) and 027 (8.2%).

Prediction rule

Seventeen variables were selected as potential predictors and included in univariate analysis (Table 1). Age, department of diagnosis, admission to an academic hospital, recent abdominal surgery, the prior use of antibiotic agents, diarrhoea as a reason for admission and hypotension were significantly associated with a complicated course of CDI after 30 days in this analysis. Sex, prior use of cytostatic or immunosuppressive agents, bloody diarrhea and Charlson's Comorbidity index, were discarded after univariate analysis due to a p-value of >0.50. The remaining twelve variables were included in multivariable logistic regression. After reduction of the model by backward selection, five variables remained strongly associated with a complicated course of CDI: age (OR 4.96 for age ≥85 years; OR 1.83 for age 50-84 years), department of diagnosis (OR 0.98 for surgery; OR 7.03 for the ICU department), recent abdominal surgery (OR 0.23), hypotension (OR 3.25) and admission because of diarrhea (OR 3.27) (Table 2). The regression coefficients of these variables were multiplied by 0.86 (shrinkage factor), after which they were converted into a score. For each patient the total score was calculated, ranging between -3 and 10. All 395 patients were stratified according to their summed score in Table 3. No patients had a summed score of >8. The observed probability to develop a complicated course due to CDI was calculated for each stratum, which showed that a high score correlated with a high risk for development of a complicated course of CDI and vise versa (Table 3).

Based on these results, four risk categories were defined: no risk (<0 points), low risk (0-1 points), medium risk (2-3 points) and a high risk (≥4 points) to develop a complicated course of CDI. A patient that is categorized in the highest group has approximately 40% chance of developing a complicated course, whereas a patient categorized in the lowest group has virtually no chance of developing a complicated

course due to CDI. After internal validation of the model, the ROC area was 0.80 for the complete risk score and 0.78 for the simplified risk score.

Table 2. Strongest, independent predictors of a complicated course of CDI in multivariable analyses.

	Odds ratio (95% CI)		P-value	Regression coefficient before shrinkage	Regression coefficient after shrinkage	Score
Age						
≤ 49 years	1	reference	reference	0.00	0.00	0
50 - 84 years	1.83	(0.68-4.97)	0.24	0.61	0.52	1
≥ 85 years	4.96	(1.40-17.6)	0.01	1.60	1.38	3
Department of diagnosis						
Other departments	1	reference	reference	0.00	0.00	0
Surgery	0.98	(0.30-3.17)	0.97	-0.02	-0.02	0
Intensive Care Unit	7.03	(2.02-24.4)	<0.01	1.95	1.68	3
Recent abdominal surgery	0.23	(0.07-0.73)	0.01	-1.47	-1.26	-3
Hypotension	3.25	(1.53-6.91)	<0.01	1.18	1.01	2
Diarrhea as reason for admission	3.27	(1.57-6.80)	<0.01	1.18	1.01	2

These predictors, selected in multivariable analyses, were included in the final model. Their regression coefficients were shrunk in order to correct for optimism and subsequently, a score was developed. The chance that an individual patient develops a complicated course due to CDI can be predicted by the following formula: p=1/(1+exp-(-3.15 + 0.52 * age50-84 + 1.38 * age≥85 + -0.02 * department of surgery + 1.68 * department of ICU + -1.26 * recent abdominal surgery + 1.01 * hypotension + 1.01 * diarrhea as a reason for admission)).

Table 3. Derivation of the risk score: predicting a complicated course of CDI.

Complete score	Patients (N)	Observed complicated course	Simplified score	Patients (N)	Observed complicated course (CI 95%)	
-3	15	0%	<0	63	0%	-
-2	40	0%				
-1	7	0%				
0	65	3%	0-1	156	5%	(2%-9%)
1	92	7%				
2	26	11%	2-3	121	17%	(10%-23%)
3	95	18%				
4	7	34%	≥4	55	39%	(26%-52%)
5	35	32%				
6	3	31%				
7	6	63%				
8	3	100%				

Using our prediction rule, several cut-off points can be used to define patients as 'at risk of a complicated course'. Sensitivity and specificity were 84% and 61% respectively for a cut-off point of ≥2, which changed to 43% and 90% for a cut-off point of ≥4. Performance of the prediction rule using different cut-off points is displayed in Table 4.

Table 4. Performance of the simplified risk score, using 3 different cut-off points to define a complicated course.

	Cut-off point for a complicated course		
	≥0	*≥2*	*≥4*
NPV	1	0.96	0.92
PPV	0.15	0.24	0.39
Sensitivity	1	0.84	0.43
Specificity	0.18	0.61	0.90
Accuracy	0.28	0.64	0.84

NPV: Negative predictive value.
PPV: Positive predictive value.

Sensitivity analyses and pilot external validation

We performed sensitivity analyses on two different patient selections: patients treated with metronidazole only and patients aged ≥15 years old (95% of the original cohort). Furthermore, we performed a complete case analysis in which 260 patients (66%) were eligible for multivariable analysis and 326 patients (83%) had complete data for the final prediction rule. All analyses yielded the same strongest five predictors of a complicated course due to CDI: diarrhea as a reason for admission, department of diagnosis, age, recent abdominal surgery and hypotension; identical to the predictors selected in the original analysis. Furthermore, similar ROC areas were found (≥0.77 in both selected patient groups and the complete case analysis).

A pilot for external validation was performed in a cohort of 139 patients. Seven of these patients (5.0%) developed a complicated course of CDI within 30 days after diagnosis. Although numbers were limited, a higher score corresponded with a higher chance on a complicated course: patients with score <0 (n=18) had 0% chance to experience a complicated course, score 0-1 (n=55) had 4% chance, score 2-3 (n=52) had 4% chance, score ≥4 had 21% chance (n=14). The risk score also performed relatively well, with an ROC area of 0.73 and a sensitivity and specificity of 43% and 92% respectively at a cut-off point of ≥4.

Discussion

In literature, *Clostridium difficile* infections are associated with high mortality risks of around 10% in the first 30 days[3, 4]. In our study, the CDI-related mortality was also 10%, and 12% of the CDI patients experienced a complicated course within 30 days after diagnosis. A complicated course was associated with advanced age, admission because of diarrhea and diagnosis at the ICU department. Furthermore, recent abdominal surgery (negative predictor) and hypotension were independent predictors of a complicated course. Here, we present a multivariable risk score for a complicated course of CDI, composed of these factors that are easily accessible at diagnosis. The score can distinguish patients with a high risk (39%) of developing a complicated course from those who have an intermediate risk (16%), low risk (5%) or virtually no risk of developing a complicated course.

Several studies previously attempted to construct a prediction rule and classify patients according to their outcome. However, none reached clinical practice due to small sample sizes and the lack of internal or external validation[13]. Two of 13 published prediction rules on the outcome of CDI were validated, however, the inclusion of subjective parameters (altered metal status) and parameters that are not available at diagnosis (radiologic findings), limited their use[23-26]. A validated risk score using recurrences as an outcome does exist[27], though its value is questioned, because it was constructed with less than 50 patients in the derivation and validation cohorts. Our prediction rule is internally validated and based on simple, clinical parameters that are available after completion of history and physical examination. This enables the physician to use it at a patient's bedside and on time for treatment guidance.

The prediction rule we present here is capable to define a high risk population: the positive predictive value rises from 12% (prevalence of a complicated course in the CDI population) to 39% when a cut-off of ≥4 is used. This high risk population is in strong need for treatment options other than metronidazole and might benefit most from novel but expensive treatments. Current evidence favours vancomycin above metronidazole in patients with severe symptoms of CDI 7, therefore, it is likely that the high risk group benefits from vancomycin. Overall, our prediction rule could guide more diverse treatment modalities, however, the exact threshold (e.g. cut-off of ≥4 or ≥2) for an treatment other than metronidazole should be determined based on careful consideration regarding the harms versus the benefits of the treatment. It should be emphasized that the majority of our patients were treated, including those with approximately no chance to develop a complicated course. This prediction rule

therefore does not confirm watchful waiting in patients with a low risk to experience a complicated course.

Advanced age has frequently been associated with mortality and a complicated course of CDI[10, 28-32]. Diagnosis at the ICU department[33] and hypotension[30, 33-35] have also been associated with a complicated course in previous research. A quarter of the patients in our study were admitted because of diarrhea, which was associated with a complicated course after 30 days. Morrison et al. found a similar percentage and association in their large cohort of 485 patients[32] and hypothesized that this could be due to a more complicated course of community-acquired infections. In our population however, 63% of the patients who were admitted because of diarrhea had been admitted to a healthcare facility in the preceding three months and were therefore not community-acquired. We hypothesize that admission due to diarrhea is a proxy for patients with severe symptoms and consequently at risk of a complicated course. Patients with recent abdominal surgery less frequently experienced a complicated course in our study. Several studies report this[10, 29, 36] and the explanation of Bhangu et al.[29] is that these patients are probably often younger and fitter compared to patients without recent surgery. This explanation seems reasonable, however, in our study the mean age (59.5 vs 61.9 years) and Charlson's Comorbidity Index (category of ≥5: 14.5% vs 17.3%) only slightly differ between patients with vs without previous surgery. Therefore, other yet unknown factors probably contribute to the difference between patients with and without recent abdominal surgery.

Serum creatinine was related to a complicated course in univariate analysis, however, it was discarded after multivariable analysis. Other laboratory parameters, such as a hypoalbuminemia and leucocytosis, were in our study not measured at diagnosis but during the course of the disease. We recently concluded that timing of these measurement highly influence the usefullness of these laboratory predictors[37]. For this reason, these potential predictors were not included in our analysis. Rapid subtyping of *C. difficile* is unavailable in most laboratories and typing data is not available at diagnosis. The presence of a hypervirulent strain such as PCR ribotype 027 was therefore not evaluated as a potential predictor in our analysis.

Although our prediction rule is constructed using strong methodology and is based on a clinically relevant outcome, our study has several limitations. First of all, the measurement of outcome is based on clinical judgement which can be subjective. To minimize ascertainment bias, outcome was based on consensus of two physicians and death within 30 days was verified by using the highly reliable Dutch National

Registration System. Although our model performed well after internal validation (ROC area 0.78) and a small external validation, its generalizability should be tested again in a setting with different researchers, locations and time. Interestingly, in our derivation and pilot-validation cohorts, the frequency of a complicated course differed (12% and 5%, respectively). Pilot-validation was done in a single center that also had a better survival during the derivation period (when 8% of the patients had a complicated course), which explains the difference.

In summary, we present a multivariable risk score that is designed to identify patients who are at risk of a complicated course of CDI. Because these patients might benefit from a different treatment, classification of patients according to their outcome could have major implications. Guidance of treatment decisions[6] and selection of high risk patients as a target population for new, but expensive, treatments may be one of the future applications[5]. Additionally, the population of different trials can now be compared and our score enables surveillances to more objectively classify patients at risk for a complicated course of CDI. External validation and determination of the clinical threshold for initiating the complicated course-treatment are aims for further research.

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Discussion

Chapter 11

A discussion

Main findings

Here, we will provide an overview of the key findings of this thesis. This overview is divided into three parts: the occurrence of CDI in two different settings; the characterization of patients at risk for CDI and a description of the disease course and outcome of CDI.

CDI emerged in hospitals and in the community

In the beginning of the 21st century, large outbreaks due to *C. difficile* PCR ribotype 027 occurred in hospitals[1, 2]. In the Netherlands outbreaks were recognized in 2005[3], resulting in the founding of a national reference laboratory. This laboratory typed and characterized *C. difficile* isolates from outbreaks and patients with a complicated course of their infection. In 2006, a surveillance was initiated in 13 hospitals to monitor the incidence of CDI. In **Chapter 2** we have described the molecular epidemiology of *C. difficile* in the Netherlands between 2005 and 2009, using samples from both the reference laboratory and the surveillance (n=2788). We concluded that *C. difficile* PCR ribotype 027 was responsible for the majority of the severe cases and outbreaks in 2005 and the first half of 2006. Thereafter, the share of type 027 decreased and three other types of *C. difficile* dominated CDI in the Netherlands: type 001, 078 and 014. After the outbreaks of CDI due to type 027, the incidence of CDI in the Netherlands remained stable at 18 per 10,000 admissions.

CDI is a notorious hospital infection, however, the infection is also increasingly recognized outside healthcare facilities. In **Chapter 3** we have summarized current knowledge on CDI in the community. Patients that develop CDI outside hospitals often (25% to 40%) have no obvious risk factors for the disease, such as prior antibiotic use or hospitalization. These patients are therefore difficult to recognize and it is unknown what predisposes them to CDI. As *C. difficile* is found in the intestinal tract of numerous animals (especially calves and piglets), the environment (such as water and soil) and meat for consumption, these sources are hypothesized to be involved in the transmission of CDI. Infection following the ingestion of contaminated meat or water seems unlikely since absolute counts *of C. difficile* spores are low and outbreaks have not been reported. Neonatal piglets primarily suffer from CDI caused by *C. difficile* type 078. As this type is increasingly associated with CDI in humans and high carriage rates are seen among farmers, circumstantial evidence points towards zoonotic transmission. However, there is currently no proof for direct transmission

of type 078 (or any other type) from animals to humans. Therefore, the incidence of CDI outside healthcare facilities is probably not driven by amplification in animals.

The classic risk profile of CDI does not apply to CDI in general practice

In **Chapter 4** of this thesis, we studied 93 hospitalized CDI patients from the Leiden University Medical Center. In this hospital CDI was endemic, with a stable incidence of 18 per 10,000 admissions. By comparing 93 CDI patients to 76 patients without diarrhoea, we confirmed that patients with a hospitalization or antibiotic therapy in the three months prior to diarrhoea had a higher risk to develop CDI. Though not significant, advanced age and underlying diseases were more frequent among CDI patients. In contrast to outbreak situations, the use of fluoroquinolones was not a risk factor for CDI in our study. As increased resistance against fluoroquinolones is seen in type 027, our results can be explained by the inclusion of a low number of patients with CDI due to this type.

Chapter 4 describes the results of a small single center study; similar data were collected in 13 Dutch hospitals and were used to evaluate antibiotic use as a risk factor in detail in **Chapter 5**. We compared CDI patients (n=337) to non-diarrhoeal controls (n=337) and showed that virtually all antibiotics increase the risk for CDI. Additionally, we showed that the risk for CDI is high when a patient is treated with an antibiotic (Odds ratio 10). This risk remains high in the first month after the antibiotic is stopped (Odds ratio 7-10). Thereafter, the risk for CDI gradually decreases: one to three months after the antibiotic is stopped, the risk for CDI decreases a fourfold, but is still increased (Odds ratio 2.5).

In **Chapter 6** we studied patients with CDI in general practice: 12,714 patients with diarrhoea and a microbiological test request from their general practitioner (not necessarily for *C. difficile*), were tested for *C. difficile*. In total, the stool of 194 patients was positive for *C. difficile* (incidence 0.67 per 10,000 person years), which was lower than *Campylobacter*, but comparable to the number of patients with a positive test for *Salmonella* spp.. Compared to matched diarrhoeal controls with a negative test for *C. difficile*, CDI patients more frequently used an antibiotic or were hospitalized before the onset of diarrhoea. These classic risk factors for nosocomial CDI, however, occurred in only 61% of all CDI patients in general practice. Consequently, 39% of CDI occurred in the absence of obvious risk factors, which may hamper adequate diagnosis of the disease.

According to data presented in Chapter 6, general practitioners detect only 40% of all CDI patients in daily routine. In our opinion, missing these patients is

undesirable, because all CDI patients included in our study visited their general practitioner because of diarrhoea and 25% of them had recurrent diarrhoea within 6 months. Furthermore, 4% of the CDI patients in our study was hospitalized because of diarrhoea following CDI diagnosis. National guidelines for the recognition of CDI outside healthcare facilities currently recommend testing for *C. difficile* in patients with diarrhoea who were recently hospitalized or used an antibiotic (19% of all diarrhoeal patients)[4]. If general practitioners followed these guidelines, the number of detected CDI patients would rise to 61%. To further increase the detection of CDI in general practice, we constructed a prediction score for CDI in general practice in Chapter 6. This score included parameters such as age, prior antibiotic use, prior hospitalization, underlying diseases and symptoms of CDI. Using this score, 44% of the patients with diarrhoea need testing to detect 85% of all CDI in general practice. Though this prediction score needs validation and cost effectiveness needs to be determined, this score could be an alternative for current testing guidelines for general practitioners.

Clinical characteristics can predict a complicated course of CDI

Together with the increasing incidence of CDI, the case fatality rate rose worldwide. In **Chapter 7** we studied the outcome of CDI in 13 hospitals in the Netherlands (n=1366). We showed that the all cause mortality risk of patients with CDI is 13% within 30 days. Although the CDI-related mortality is difficult to estimate because the mortality risk is associated with underlying diseases that predispose for the infection, we observed that the 30-day mortality rate of CDI patients (n=317) was 2.5 times higher compared to similar controls without diarrhoea (n=317). CDI-related mortality occurred mainly within 30 days after diagnosis. The high mortality rate occurred in a population where 90% of the CDI patients was treated for CDI, which highlights the need for alternative treatment options.

It is difficult to distinguish patients who will respond to treatment and are subsequently cured, from those who develop a complicated course (e.g. treatment failure or death). Selecting predictors of a complicated course could help physicians to recognize these patients and, eventually, optimize treatment in this group. The European Society of Clinical Microbiology and Infectious Diseases listed multiple putative markers for severe disease in a treatment guidance document[5]. In **Chapter 8** we investigated if three of these markers could adequately predict treatment failure. Among 1105 patients that participated in a randomized controlled trial, fever (temperature >38.5°C), renal failure (creatinine count ≥133 mmol/L)

and leukocytosis (leukocyte count >15*10^9/L) were significantly associated with treatment failure (persistence of diarrhoea or need for additional CDI treatment). Using a cohort of 104 hospitalized adults with sequential recorded laboratory parameters (±3 days of diagnosis), showed that creatinine and leukocyte counts were highly variable around the day of diagnosis. Therefore, leukocytosis and renal failure could be useful predictors of treatment failure, although these parameters need strict definitions concerning the timing of the measurement. Fever occurred in only 1% of the CDI patients, which limits the clinical value of this potential predictor.

In **Chapter 9** we investigated the association of a bacterial virulence marker (binary toxin) and the 30-day mortality rate. In contrast to the selection of predictors in Chapter 8, this study has an etiologic aim. Binary toxin is often found in *C. difficile* isolates that cause severe disease or a complicated infection[6] and this toxin is speculated to improve bacterial adherence and colonization of the gut[7]. To investigate the role of binary toxin as a cause of a complicated course, we studied the association of binary toxin and mortality in a large population (n=1366). The analysis of binary toxin positive strains was stratified according to PCR ribotype: type 027 strains and non-027 strains. Type 027 was associated with a higher 30-day mortality compared to patients with a binary toxin negative strain (22% vs 11% 30-day mortality; HR 2.2, 95% CI 1.2-2.4). Patients with a binary toxin positive strain other than type 027 died only slightly more frequently than patients with a binary toxin negative strain (15% vs 11% 30-day mortality; HR 1.5, 95% CI 0.8-2.6). Currently there is no convincing evidence that binary toxin causes a high 30-day mortality.

In **Chapter 10**, we constructed a prediction score for a complicated course due to CDI. A complicated course was defined as an ICU admission, colectomy or death due to CDI within 30 days after diagnosis. Among 395 CDI patients from 13 Dutch hospitals, we selected putative predictors that were available at the patient's bedside at time of diagnosis. Age, admission due to diarrhoea, diagnosis at the ICU department, hypotension and recent abdominal surgery were predictors of a complicated course. By including these predictors in a prediction model, we were able to classify patients according to their risk for CDI: high risk (39% with a complicated course), intermediate (16%), low (5%) or virtually no risk to experience a complicated course. This prediction score was externally validated in a small cohort.

CDI treatment is currently not very heterogeneous, and most CDI patients are treated with metronidazole (Chapter 6). As more treatment options are available, classifying patients according to their outcome could potentially guide treatment decisions.

Methodological considerations

Before putting our main findings to perspective, three methodological issues will be discussed, that need to be considered in research on *Clostridium difficile* infections. Apart from highlighting these issues in the current thesis, we will give examples from international research. We will use this consideration to propose recommendations for the design of future research (further on in this Chapter).

Design – why and how do we use case-control studies?

In principle, a valid and transparent design to determine risk factors or predictors of nosocomial CDI would be a cohort[8]. In this design, patients (exposed and unexposed) are followed over time while the outcome occurrence is closely monitored. Finally, the risk for the outcome is determined by comparing exposed and unexposed patients. In CDI research for example, all consecutive hospitalized patients are included during the study period whereafter the risk for CDI is measured by comparing patients with and without antibiotic use.

CDI is relatively common in hospitals, however, only 1 in 500 hospitalized patients develops the infection. In order to gain enough power, cohort studies concerning risk factors for CDI require a large timeframe, a large sample size or high incidence of CDI. Consequently, cohort studies in CDI research are mainly used during large outbreaks[9] or when large computerized datasets are available[10]. When the outcome under study is relatively rare (CDI in this case) or large data gathering make this design impracticable, a case-control design can be chosen[11]. This efficient design is popular in CDI research as it includes all patients with CDI and only a selection of the patients without CDI.

The main challenge when designing a case-control study is the appropriate selection of controls. Controls should represent the population from which cases are derived. An example of a well chosen control group is the study of Dial et al. concerning risk factors for CDI in the community[12]. Among 3 million people who were registered in the United Kingdom General Practice Research Database, patients with a first episode of CDI were selected as cases. Per case, 10 controls without (prior) CDI were selected from the same database. Of both cases and controls data regarding proton pump inhibitor (PPI) use were available in the computerized dataset. A less well chosen control group was used in an English study that also aimed to determine risk factors for CDI in the community[13]: among patients who visited a general practitioner, CDI patients were compared to patients with diarrhoea and a negative

laboratory test for CDI. Patients with diarrhoea do not represent the total population at risk for CDI, in which most patients will not have diarrhoea. As a result, risk factors for diarrhoea with a negative test for CDI (e.g. antibiotic use) can not be investigated for their causative role in CDI. A second study without a representative control group was conducted in North America: CDI patients detected in the hospital laboratory (both hospitalized and outpatients) were compared to a group of randomly selected outpatients[14]. Because CDI patients included hospitalized and outpatients, controls derived from outpatients solely are a poor representation of the source population (e.g. all patients visiting the hospital or all patients in the catchment area of the hospital). Again, risk factors for being an outpatient (e.g. underlying illnesses) can not be investigated for their role in CDI.

In the present thesis, we also encountered difficulties with control group selection. In Chapter 4, 5 and 7 two control groups were selected to identify risk factors for nosocomial CDI: one consisted of patients with diarrhoea and one consisted of patients without diarrhoea. It has been reported that findings may be more trustworthy when they are consistent in two different control groups, however, when opposite results occur it is unclear what finding to believe[15]. Therefore, a single control group is often recommended. In Chapter 4, 5 and 7 a single control group of randomly selected hospitalized patients, would have been a good alternative for our two control groups. By using this single control group (without taking the presence of diarrhoea into account), we would have obtained a more appropriate selection of the source population since all hospitalized patients could be included. As in the aforementioned English study, the selection of diarrhoeal controls limits the risk factors that can be studied. In the present thesis, we considered the results of the control group without diarrhoea as the most valid, since this control group represents the population from which the cases are derived best (hospitalized patients) and enabled us to study most risk factors for CDI.

In Chapter 6, CDI patients were compared to other diarrhoeal patients that visited a GP. This control group is suitable to select predictors for CDI amongst patients with diarrhoea, which is the main aim of Chapter 6. Conclusions regarding the etiologic function of these predictive factors should however not be drawn, as the control group was not a sample from all patients at risk for CDI (e.g. all patients who could visit these general practitioners). For example: the use of PPIs was not significantly associated with CDI in Chapter 6. There might be no effect of PPIs on CDI, however, when PPIs are associated with both CDI and diarrhoea due to other causes, an association can be obscured. Recent meta-analyses show that the use

of PPIs is associated with a higher risk on CDI[16-18]. As literature also shows that PPIs are associated with both CDI and bacterial enteritis due to e.g. *Campylobacter spp*[19, 20], a control group of diarrhoeal patients is clearly insufficient to study PPIs as a risk factor for CDI. With this aim, selecting population controls by e.g. random patients selection from the GP patient database, is more suitable.

In conclusion, the case-control design is frequently used in CDI research and has major benefits regarding efficiency and costs without necessarily compromising on the validity of the study's conclusion. This only holds when appropriate controls are used; poor choice of controls can lead to biased results.

Misclassification

Diarrhoea and the presence of toxin producing *C. difficile* are the twin pillars for CDI diagnosis. Multiple laboratory tests with different targets (toxins, toxin genes, enzymes, the *C. difficile* bacterium) are available but all have either limited sensitivity or specificity[21]. Misclassification of CDI patients and diarrhoeal patients without *C. difficile* are therefore potential pitfalls for CDI research.

In this thesis, several laboratory tests were used to diagnose CDI, including an enzyme immunoassay (EIAs) to detect faecal toxins. These tests are frequently used in CDI research, relatively cheap and specific (98%), but lack optimal sensitivity (70-90%)[22]. In Chapter 8 and 9 of this thesis, an EIA was used to select a cohort of CDI patients. Due to the limited sensitivity of EIAs, false negative patients could have occurred. According to a small American study (n=132), EIA negative patients have similar characteristics and outcomes as patients with a positive test (both were treated)[23]. Therefore, in our cohorts of CDI patients, missing patients due to poor test sensitivity probably not largely influenced our results. In Chapter 4, 5, 6 and 7 of this thesis, a case-control design was used to study prognostic markers or risk factors for CDI. In this design, poor test sensitivity not only results in missing cases but also makes false negative patients suitable for selection as (diarrhoeal) controls. As most false-negatives will not be sampled as controls (large sampling fraction), misclassification due to non-recognition of cases usually does not result in large bias. In Chapter 7 this statement holds. In Chapter 5, 6 and 8, however, a diarrhoeal control group was selected. As diarrhoeal patients were scarce, false negative CDI patients had a relatively high chance to be included as controls. Misclassification of false-negative patients might therefore have caused bias when comparing CDI patients to diarrhoeal controls (dilution of the effect). In our thesis, we report the

comparison of CDI patients to controls without diarrhoea as the most valid. These conclusions cannot be influenced by inclusion of false-negative cases as controls.

Although different testing regimens are applied in hospitals worldwide, *C. difficile* is detected in about 10% of the hospitalized patients with diarrhoea[5]. Due to a relatively high **specificity** of most tests[24], including e.g. EIAs, the positive predictive value in a hospital setting is around 80%. In Chapter 7 we describe CDI in general practice where the prevalence of CDI is around 1-2%. In this setting, false positive results are of concern, as positive predictive values can be less than 50%, even with a relatively specific test. Consequently, patients with diarrhoea and a false positive test are misclassified as CDI cases.

In conclusion, false negative results – although frequent in CDI research – do not necessarily constrain study validity when controls consist of a small fraction of the source population. Misclassification due to false positive results is of greater concern, especially in environments with a low prevalence of CDI, because all positive results are included as cases (no sampling fraction).

Problems with sensitivity and specificity can be overcome using a single perfect diagnostic test. As such test is currently not available, CDI research could benefit from a two-step test algorithm combining a sensitive screening test with a specific confirmation test in the diagnosis of CDI[24, 25]. Molecular tests on toxin genes or EIAs targeting the enzyme glutamate dehydrogenase (GDH), which is produced by all *C. difficile* organisms, are often mentioned as options for a first sensitive screening test. Both tests have a sensitivity above 90%[24]. Although EIAs to GDH can detect *C. difficile*, they do not discriminate between isolates that are capable of producing toxins and non-toxigenic isolates. Molecular tests on toxin genes have the advantage to detect only *C. difficile* isolates with toxin genes. However, these tests do not discriminate between CDI and asymptomatic colonization with *C. difficile*, as they do not detect faecal toxin[26]. Both molecular tests and EIAs on GDH therefore need to be followed by a test that detects faecal toxins (e.g. EIA on toxins) according to a recent guideline in the United Kingdom[27].

Although European and American guidelines recommend this two-step testing, combining multiple tests for CDI diagnosis is costly and this algorithm is not yet widely implemented in CDI research. To limit misclassification due to false positive results, some studies tried to confirm their initial positive result by culture of *C. difficile* and subsequent typing, whereafter they present a restricted analysis of cases with confirmed CDI[28]. However, most studies focus on the low sensitivity and forget to

mention the possibility of false positive results due to insufficient specificity[29, 30]. Future research should pay more attention to this potential flaw.

Defining the outcome of CDI

C. difficile can cause diarrhoea, pseudomembraneous colitis, septic syndrome or death, even despite treatment (Chapter 7). In CDI research, the outcome of CDI is often reported to show the benefit of an intervention (in e.g. a randomized controlled trial), to describe the natural history of CDI or to divide *C. difficile* strains according to their virulence. Multiple different outcomes are used in CDI research and in our thesis (Table 1).

Table 1. Outcomes used in the studies in this thesis.

Outcome	Definition	Chapter
Clinical failure	persistence of diarrhoea or the need for additional CDI therapy, or both on the basis of the opinion of the investigator	8
Recurrence of CDI	>3 unformed bowel movements per 24 hours and a positive stool toxin test result during follow-up	8
Complicated course	ICU admission or colectomy due to CDI, death within 30 days	4
Complicated course due to CDI	ICU admission or colectomy or death within 30 days, all due to CDI	10
Severe diarrhoea	bloody stools, hypovolaemia, fever (T>38.0°C) and leukocytosis (>12.0*10^9/l), hypo-albuminaemia (<20g/l) or pseudomembranous colitis	4
Death	death within 6 months death within 30 days	6 4, 7, 9

As heterogenic outcome definitions make studies difficult to compare, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID)[31], the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA)[32] formulated recommendations for uniform outcome assessment in surveillances. Surveillances often collect outcome data on CDI patients only, disregarding CDI negative patients. To report the influence of CDI on mortality, both guidelines recommend assessing the outcome as 'complicated course due to CDI' (as we used in Chapter 10) in addition to the all cause mortality. In this definition, the contribution of CDI to death, ICU admission and colectomy are included.

Although the recommended definition seems straightforward, the contribution of CDI to death is deemed to be subjective. To limit bias by this subjective ascertainment, some studies asked two or more physicians to agree whether CDI contributed to the outcome. Two important studies in the field of CDI used

this approach to measure their outcome[1, 33]. In addition, the inter-observer ratio can be used show the level of agreement between the physicians and therefore the precision of the measurement. However, the CDI-related mortality remains a subjective outcome and an approximation is always needed.

Besides surveillance studies, subjective outcomes are used in some etiologic studies in CDI research. In clinical trials, including the two trials of Chapter 8, patients with life-threatening CDI are often excluded. Therefore, mortality is not expected to occur and the all cause mortality is not included as an endpoint. As an alternative (or even first choice outcome) the clinical failure of CDI was defined in Chapter 8. This definition included 'resolution of diarrhoea' or 'no need for CDI treatment according to the treating physician', which is again slightly subjective.

Although surveillance studies can benefit from the definition of a complicated course of CDI, we prefer the use of an outcome less debatable for most study designs (including clinical trials that estimate treatment effect). According to a recent systematic review, the most frequently used outcome in etiologic studies concerning CDI is all cause mortality (after 30, 60 or 90 days)[34]. This outcome can hardly be misclassified and is suitable in many etiologic and prognostic studies in CDI research. In Chapter 7, we used 'all cause mortality within 30 days' when calculating if CDI influenced the outcome of infected patients. In Chapter 10, we searched for predictors for an unfavorable outcome of CDI. In CDI research, both the 'all cause mortality within 30 days' and a 'complicated course due to CDI' are used to select predictors for an unfavorable outcome of CDI[35]. Although some prefer an objective measurement, the latter could be of benefit when searching for specific predictors associated with CDI outcome. As patients with a high risk of a CDI-related mortality might benefit from a different treatment (more on this topic can be found in the recommendations for future research), selection of predictors associated with CDI can, in our opinion, be useful.

In summary, many different outcomes are currently used in CDI research. Especially in surveillance studies a less diverse spectrum is preferable to enable comparison of study results. The complicated course due to CDI is a valid option, In most other study designs we prefer a more objective outcome measurement such as the all cause mortality.

Findings in perspective

In the present thesis we have described the current clinical spectrum of CDI: its occurrence, the population at risk, course and outcome of CDI in different populations. In each chapter we discussed our findings and the relevant existing literature. Now we would like to present an integrated view of the findings in our thesis, in light of the current knowledge about the evolution of CDI in time and the recent changes in diagnostics and therapy of CDI.

Nosocomial CDI, still an underestimated infection

Following multiple outbreaks of CDI worldwide, the incidence of nosocomial CDI showed a less steep increase in many countries after 2008[36]. The Netherlands, Belgium, Finland and the United Kingdom even reported a stable or declining incidence (Chapter 2)[37-40]. Currently, CDI occurs in 18 per 10,000 hospital admissions in the Netherlands, which is 10 times lower than the endemic incidence in the United States of America[41] but comparable to the incidence in the United Kingdom[37] and the rest of Europe[42]. Compared to nosocomial Methicillin-resistant *Staphylococcus aureus* (MRSA), which is another frequently encountered bacterium in hospitals, nosocomial CDI now exceeds its incidence[43, 44]. Compared to MRSA bacteraemia, CDI was ten times more likely to occur in hospitals in the United Kingdom[37]. This highlights CDI as an important hospital associated infection.

The reported incidence is based on the occurrence of CDI; but as the diagnostic algorithm, the awareness of physicians and the availability of a national surveillance also influence this measurement, the incidence of CDI is currently underestimated in most European countries, including the Netherlands[42]. A recent European study revealed suboptimal diagnostic procedures for CDI[45]: although 95% of 126 hospital laboratories from 31 countries had CDI tests available, one third used a single test with limited sensitivity (most frequently an EIA on toxins) for CDI diagnosis while an algorithm of multiple tests is the preferred method according to recent guidelines[25]. These results are also seen in Dutch hospitals: a third currently uses an EIA on toxins as a first test[46]. In the Netherlands, most laboratories test all diarrhoeal stool samples from hospitalized patients. In other European countries, however, comprehensive testing is not applied and the incidence of CDI depends on the awareness among healthcare professionals to request a test. According to a 1 month pan-European surveillance, the frequency of testing for nosocomial CDI varied up to 47 times among the 34 participating countries[42]. National awareness for

CDI can increase with active hospital surveillance. This is present in many European countries; in the UK, Germany and France surveillance is even mandatory for severe cases of CDI or outbreaks[47]. In the Netherlands, 18 hospitals participate in an ongoing voluntary national surveillance[46]. Based on the three aforementioned parameters that influence the incidence of CDI, we expect the incidence of CDI to rise in the near future. Although CDI is stably present in the Netherlands and the awareness of nosocomial CDI is relatively high, the introduction of new diagnostic algorithms, mostly based on molecular diagnostics with a high sensitivity, will increase the detection of CDI in the Netherlands[26].

Molecular epidemiology reveals that CDI is dynamic

Together with the stabilizing incidence of CDI, the molecular epidemiology changed. In 2005, type 027 predominated in the Netherlands but was in time replaced by types 001, 078 and 014 (Chapter 2). Similar changes were observed in Europe between 2007[48] and 2011[42]. According to a 10-year lasting surveillance study, the occurrence of *C. difficile* types is dynamic: types that cause outbreaks become endemic after some time, while other types emerge[49]. As outbreaks due to type 027 became less frequent in many countries, it was likely that other types emerged. In the Netherlands, type 078 was found emerging[50], while in England various types (002, 015 and 078) increasingly caused CDI[51, 52].

Although other strains became prevalent, outbreaks were found sporadically and by far not as widespread as those seen with type 027. Furthermore, types 002, 015 and 078, were infrequently associated with a complicated course[50, 53]. Whole genome sequencing or sequencing a small part of the genome with 'multi-locus sequence typing', can divide *C. difficile* isolates into five and six 'clades', respectively[54-56]. These clades are formed by isolates with a similar genomic evolution, which suggests a similar behavior in patients. Type 002 and 015 belong to clade 1, type 027 to clade 2 and 078 to clade 5[54]. According to two recent studies among 2299[6] and 2222[57] CDI patients, CDI caused by type 078 was associated with a high short term mortality, which was comparable to the mortality of CDI due to type 027. The latter study even concluded that not only 078 and 027 were associated with a high mortality risk, but all strains in their clades. Not all studies, however, confirmed this higher mortality risk in CDI with type 078. Chapter 7 and 8 of our thesis (n=1366) confirm that type 027 is associated with a high mortality in the first 30 days after diagnosis, but mortality among patients with CDI due to type 078 turned out to be not significantly higher. In two other studies this association was also lacking[50, 58]. The

different conclusions concerning the mortality of patients with type 078 might be explained by the numerous differences in design: different testing methods, patient selections, outcomes and (insufficient) adjustments for confounding[59]. Furthermore, in all studies conclusions were based on fewer than 100 patients with CDI due to type 078.

The infrequent association of a complicated course and less extensive outbreaks caused by currently circulating types could be a result of the improved treatment and prevention measures during outbreaks but might also be a result of differences in bacterial factors. As we listed in Chapter 1 many bacterial factors have been implicated to contribute to virulence, including increased toxin production[60], sporulation[61], colonization[7], evasion of the immune system and increased antibiotic resistance[62]. Similar to type 027, types 002, 015 and 078 produce toxin A and toxin B. Like type 027, type 078 is binary toxin positive and contains a deletion in the *tcdC* gene. Differences, however, are also present: fluoroquinolone resistance was associated with the extensive spread of type 027 according to a recent paper that studied the emergence of type 027[62]. This resistance is infrequent in type 078[63]. Furthermore, type 027 seems to have a higher 'infection to colonization' ratio, in comparison to other *C. difficile* isolates[33].

In conclusion, the molecular epidemiology of *C. difficile* shows that CDI is dynamic. Until it is clear what the exact virulence factors of *C. difficile* are and which types contain these factors, the emergence of types of *C. difficile* should be monitored to detect new (hyper)virulent types in time and to prevent extensive spread.

CDI awareness lacks outside hospitals

The relatively stable situation of CDI in Dutch hospitals is overshadowed by major outbreaks in nursing homes. According to data from the Dutch reference laboratory for *C. difficile*, nursing home patients are currently a large source of type 027 in the Netherlands[46]. Between 2009 and 2012, two major outbreaks occurred in nursing homes involving at least 60 patients and accompanied by high mortality rates[46]. In nursing homes, awareness and diagnostics of CDI (and therefore also treatment and prevention) are not yet widespread[64], which caused late recognition and extensive spread of the infection. In the community, awareness is also low and testing is inconsistently applied; consequently, many cases are missed (Chapter 6).

Although often seen as two different entities, recent publications suggest transmission of infecting strains from nosocomial to community-associated CDI and vice versa. The majority of the CDI cases in nursing homes occur within 30 days after

hospital discharge[65, 66] and it is speculated that many hospitalized patients become infected following contact with an asymptomatic carrier from the community[57]. The latter was concluded in a study from Oxford in which *C. difficile* genomes of 486 hospitalized CDI patients were sequenced. It was shown that patients in one hospital were infected with many different strains, which made transmission between symptomatic patients or their environment as the prime source of infection unlikely. The authors therefore speculated that asymptomatic carriers of *C. difficile* are an important source of infection in hospitals[67]. Former studies that tried to elucidate the transmission of nosocomial CDI used PCR ribotyping or Multiple-Locus Variable number tandem repeat Analysis (MLVA); the present study used whole genome sequencing for its analysis. As this is the first study using this highly discriminative technique, future research should confirm its findings. As transmission of *C. difficile* seems to occur between settings, CDI detection and prevention should be widely applied in order to further diminish CDI burden in the Netherlands. Additionally, the risk that is associated with transmission of *C. difficile* from asymptomatic carriers should be further investigated.

Risk factors for CDI in the community need to be elucidated

As was extensively studied in healthcare facilities, well known risk factors for CDI are prior hospitalization, antibiotic use and severe underlying diseases[68]. These factors are present in virtually all CDI patients in the hospital. In Chapter 4 and 5 of this thesis, we confirmed the presence of these risk factors in an endemic hospital setting.

In the community, recent hospitalization or antibiotic are absent in over one third of the CDI patients (Chapter 3 and 6). Currently, it is unknown what makes these patients susceptible to CDI and where they acquire *C. difficile*. As we state in Chapter 3, literature review does not provide evidence that CDI in the community is driven by zoonotic transmission: direct transmission was never proven and frequently found PCR ribotypes (e.g. 001, 027 and 014) do not en mass occur in a suggested zoonotic source. In Chapter 3 we also state that PCR ribotype 078 could have zoonotic potential, but this contribution to CDI in the community is likely to be small. Data from the study of Chapter 6 strengthen this conclusion, as contact with piglets was only sporadically found among patients with CDI in general practice (7%; unpublished data from the study in Chapter 6). In The Netherlands, piglets with CDI are infected with type 078 only, whereas type 078 is responsible for 'only' 9% of the nosocomial CDI (Chapter 2) and 10% of the CDI in general practice (Chapter 6).

If zoonotic transmission is not the driving force for CDI outside healthcare facilities, what causes infection in this setting? Some suggest that transmission of *C. difficile* to susceptible patients is facilitated by young children[13], whereas others suspect the environment or healthy individuals who carry toxigenic *C. difficile* (Chapter 3). Future research should determine what risk factors other than underlying diseases and proton pump inhibitors[16-18] make patients in the community or general practice susceptible to CDI.

As risk factors for CDI in the community are largely unknown, CDI patients are hard to distinguish from other diarrhoeal patients in general practice, based on clinical information (Chapter 6). Although we advocate consideration of CDI in all diarrhoeal patients for whom a pathogen is warranted (Chapter 6), a drawback of this recommendation is that testing all these patients is costly and diagnostics for CDI are currently too insensitive to test all patients in whom CDI is considered. Future research should therefore focus on optimization of (multiple-step) testing algorithms. Meanwhile, considering CDI in only patients with a high risk profile is an option. According to Chapter 6, elderly patients with antibiotic use and severe diarrhoeal complaints have the highest risk for CDI. We propose a prediction score, that includes these parameters. However, future research should continue to search for other or better predictors for CDI outside hospitals, as still 40% of the diarrhoeal patients need to be tested with the current score to detect 85% of the CDI patients.

In contrast to the population of CDI patients in the community, known risk factors for CDI are frequently seen among patients in nursing homes[69]. Consequently, the infection is frequent[70]. Testing all patients with diarrhoea in a nursing home is not (financially) achievable in many Dutch nursing homes[64, 69]. Therefore, identification of patients at high risk should preferably be used to guide testing. Many nursing home residents fit a high risk profile of old age, recent antibiotic use or underlying diseases, which makes patients with an increased risk for CDI difficult to recognize. A prediction score for CDI to support nursing homes physicians in recognition of CDI and to advise physicians on testing patients at high risk for CDI is therefore an aim for future research.

The outcome of CDI – treatment of infected patients

Clostridium difficile infections have a major impact on healthcare costs and patient morbidity as they are associated with prolonged hospitalization, inter-patient spread and medical complications[1, 2, 71] (Chapter 1). An obvious way to combat the healthcare implications of CDI is prevention of the infection. Prevention includes

early diagnosis and surveillance of CDI, education of the staff, isolation precautions, hand hygiene, protective clothing, and cleaning of the environment and medical equipment[72]. Additionally, the restriction of certain antibiotic classes (good antibiotic stewardship) can reduce the susceptibility of patients for CDI. In the Netherlands, an outbreak of type 027 was ended after restriction of cephalosporins and a complete ban of fluoroquinolones in addition to regular prevention measures[73]. The Dutch national institute for Public Health and the Environment (RIVM) provides guidelines on the prevention of CDI[74, 75] and started a national surveillance for CDI among approximately 20 hospitals. Hospitals included in this national surveillance keep track of the incidence and molecular epidemiology of CDI, clinical characteristics and outcome of patients and they have access to annual educational workshops. In England, a mandatory surveillance for CDI and a national target for the reduction of the infection were introduced[52]. This target was set by the English Department of Health, and aimed to reduce the number of CDI cases with 30% within three years. So called improvement teams intervened in institutions that did not meet the pre-specified target. At the end of this three year period, the aim was exceeded and currently, numbers of patients CDI still decrease.

When CDI occurs despite preventive measures, the majority of the CDI patients (75%) is treated with metronidazole, another 15% receives vancomycin or a combination of both (Chapter 7). Many more treatments are available or currently tested in phase 3 studies. According to randomized controlled trials, treatment of a first episode of CDI may also include administration of monoclonal antibodies against *C. difficile* toxins or the macrocyclic antibiotic 'fidaxomicin'. Adding monoclonal antibodies against toxin A and B to standard antimicrobial therapy significantly reduced recurrence rates of CDI according to a recent trial among 200 in- and outpatients[76]. In another trial, fidaxomicin had cure rates similar to vancomycin but excelled in lower recurrence rates, which were seen in 15% and 25% in the fidaxomicin and vancomycin group, respectively[77]. This benefit might be a result of the selective eradication of *C. difficile*, while keeping the intestinal flora intact. Of note, this beneficial effect seems absent in CDI caused by type 027. Although both monoclonal antibodies and fidaxomicin have potential benefits, treatments are costly and in case of fidaxomicin no information on development of resistance is currently available. When recurring CDI occurs, patients might also benefit from fidaxomicin or monoclonal antibodies. However, these patients could also benefit from the infusion of healthy donor feces[78]. A recent trial that compared this relatively simple therapy with vancomycin treatment, was prematurely ended due

to the significantly higher success in the faecal transplantation group (81% vs 31% resolution of diarrhoea without relapse). The rationale behind the success of donor faeces is the rapid restoration of the gut flora. Patients with CDI have less diverse gut flora, with changed relative proportions of two frequently found bacterial phyla in the gut (relatively less *Bacteroidetes* and more *Firmicutes*)[78, 79]. This changed composition is hypothesized to form a niche for *C. difficile* to flourish. According to the recent trial with faecal transplantation, the infusion of donor faeces from a healthy individual increases the diversity of the gut flora and restores the changed proportions of *Bacteroidetes* and *Firmicutes*. These beneficial changes persisted during the 10 weeks follow-up[78]. As a disturbed gut flora is regarded as a major risk factor for CDI, several trials tried to prevent CDI by probiotic treatment. Although the beneficial effect of probiotics was doubted for many years, a recent meta-analysis among 20 randomized controlled trials concluded that there was evidence towards a (strong) beneficial effect of probiotics[80]. However, this conclusion was based on moderate quality evidence according to the authors of the meta-analysis. To date, the benefits of toxin binders or other antibiotics such as tigecyline, have not been proven in a randomized clinical trial.

Among hospitalized patients with CDI, mortality risks are high despite treatment. According to Chapter 7 of our study, a large Canadian cohort[81] and many other studies[1, 2, 33, 82], the CDI-related mortality risk is around 10% in the first month after diagnosis. To move from this group specific mortality risk to an individual risk prediction, we searched for predictors for a complicated course of CDI and evaluated them in a risk prediction model (Chapter 8 and 10). According to our studies and recent literature, predictors of a complicated course are often general markers for inflammation (leukocytosis, CRP, fever) or general welfare of a patient (albumin, severe underlying diseases, renal failure)[35]. Additionally, IgG to toxin A[83], *C. difficile* PCR ribotype (Chapter 9)[57] and abnormal findings on a computed tomography scan[84] were identified as specific predictors for complicated CDI. To use a biomarker in a prediction model for complicated CDI at diagnosis, the result should be available in time. Currently, PCR ribotyping is performed after culture of *C. difficile*. As culture takes a minimum of 2 days, the results are currently too late for inclusion in a prediction model at diagnosis. Rapid typing methods such as specific PCRs for e.g. PCR ribotype 027 in faeces could enable inclusion of this predictor in a model in future[85]. IgG to toxin A and computed tomography scans are not widely applied in and tested for in patient care, which makes them unsuitable for risk prediction among CDI patients.

Both PCR ribotype and laboratory biomarkers such as white blood cell count (WBC count), albumin and C-reactive protein (CRP) were predictors of mortality in the previously mentioned study from Oxford, performed between 2006 and 2011[57]. Although strain type was an independent predictor for mortality when combined with biomarkers in this study, a study by Walk et al. concluded that PCR ribotype did not add to laboratory predictors in predicting the outcome of CDI[58]. In addition to Walk et al., more and more people regard clinical biomarkers instead of bacterial biomarkers as the most promising predictors of a complicated course[59, 86].

Recommendations for future research

Apart from the recommendations that we have already made in the preceding part of this Chapter, we will now propose two points of particular interest for future research in the field of CDI: It is important to find out if *C. difficile* carriers, who are numerous, can cause spread and infection with *C. difficile.* This could have major implications for e.g. the prevention of the disease. Second, identifying patients at risk for therapy failure could change the management of CDI in future.

Hospitalized CDI patients have well described risk factors, including prior antibiotic use, underlying illnesses and infection pressure (exposure to infected patients or their environment). Although a study from 1992 suggested that contact with asymptomatic carriers of *C. difficile* formed a risk factor for the disease, it is common belief that (in)direct transmission of *C. difficile* from symptomatic patients in the hospital is the major source of nosocomial infection. Infection control and diagnostics are therefore directed at symptomatic patients only[72]. Recently, several studies again highlighted the potential risk that is associated with asymptomatic carriage of *C. difficile*[67, 87, 88]. Carriers of *C. difficile* are numerous: in Canada, 4% of all admitted patients are carriers of *C. difficile* on admission; an additional 3% become carriers of the bacterium during hospitalization[33]. Furthermore, 2% of adults in the community are estimated to carry *C. difficile*[89]. Recent developments in diagnostics such as the development of a PCR targeting *C. difficile* toxin genes, enables us to detect carriers of toxigenic *C. difficile*. Additionally, the use of whole genome sequencing to discriminate *C. difficile* strains with high resolution, make it currently possible to thoroughly investigate the role of asymptomatic carriers in the transmission of *C. difficile*. If transmission of *C. difficile* is mediated by *C. difficile* carriers, this could have major implications for prevention, diagnostics and treatment. In our opinion,

future research should be directed at solving this issue as this could influence the burden of CDI worldwide.

A second recommendation we want to make is directed towards the choice of treatment of CDI. Many studies, including ours, focus on the selection of predictors of a poor outcome of CDI to subsequently include the predictors in a prediction score. The aim of these prediction scores is uniform: to identify patients at high risk for a poor outcome, as high risk patients might benefit from enhanced treatment[83, 90, 91] (Chapter 10). It is tempting to believe that patients that benefit most from newer or more expensive treatment options are those at high risk for a complicated course. However, this remains to be proven in the case of CDI[92]. Subgroup analyses in randomized clinical trials hint towards a better performance of vancomycin over metronidazole in patients with 'severe CDI'[93]. Fidaxomicin seems to reduce relapses among patients with CDI due to a non-027 strain better than vancomycin in a fase 3 clinical trail[77]. However, subgroup analyses according to a validated prediction score have not been done. It is therefore unclear if a high prediction score is associated with a beneficial treatment response besides a poor prognosis[94]. Apart from a subgroup analysis within a clinical trial by stratifying according to the prediction score, a new randomized trial preferably should confirm the value of heterogeneous treatment options based on a prediction score.

Besides proving the added value of heterogeneous treatment options, the cost effectiveness should be determined. Enhanced treatment options for CDI (vancomycin and fidaxomicin) are well tolerated and have limited side effects. Therefore, the main issue currently consists of high treatment costs. To evaluate the cost effectiveness of a treatment guide that is based on a prediction score, this algorithm should be compared to current treatment without knowledge of the prediction score[94].

To date, no efforts have been made to evaluate and introduce stratified medicine according to a prediction score in CDI research. This is therefore, a major challenge for future research.

Conclusion

This thesis offers insight in the epidemiology of *Clostridium difficile* infections. As we highlighted in the introduction, the first aim of this thesis was to characterize patients at risk for CDI in more detail and consequently contribute to the recognition

of CDI. This aim was investigated in both general practice and in a hospital setting. We confirmed the presence of classic risk factors for CDI in an endemic hospital setting (Chapter 4), identified new risk factors, such as antibiotic use in the preceding 3 months (Chapter 5), and identified predictive factors for CDI, e.g. severe complaints (Chapter 6). Therefore, this thesis might contribute to the recognition of CDI, which was an indirect aim of this thesis. A second aim was to recognize factors that are associated with a complicated course and outcome of CDI. Besides providing an overview of the course of CDI in the Netherlands (Chapter 7), we characterized patients with a complicated course of CDI (Chapter 7, 8, 9, 10). As this information might help physicians to identify patients at risk for deterioration and failure of therapy, we have contributed to our second aim.

The results of this thesis might, in the future, contribute to patient counseling and treatment guidance.

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Chapter 12

Dutch summary
Nederlandse samenvatting

Ziek door de *Clostridium difficile* bacterie

Dit proefschrift gaat over patiënten met een darminfectie door *Clostridium difficile*. Deze bacterie kan toxinen produceren die schade aan de darm geven en daardoor een *C. difficile* infectie (CDI) veroorzaken. Het proefschrift bestaat uit 4 onderdelen: een inleiding waarin een overzicht wordt gegeven van de problematiek die *C. difficile* op dit moment veroorzaakt (Hoofdstuk 1, 2 en 3); een onderdeel dat beschrijft welke mensen er ziek worden door *C. difficile* (Hoofdstuk 4, 5 en 6); een onderdeel waarin de ernst van de infectie wordt beschreven (Hoofdstuk 7, 8, 9 en 10) en een laatste deel waarin de bevindingen van dit proefschrift worden bediscussieerd (Hoofdstuk 11).

Inleiding

Een infectie door *Clostridium difficile* geeft klachten als buikpijn, koorts en (bloederige) diarree, vooral tijdens of na het gebruik van antibiotica. Ouderen en mensen met onderliggende ziekten hebben een verhoogd risico op de infectie. Tot 2000 stond CDI niet erg in de belangstelling, maar vanaf het begin van de 21^{e} eeuw ontstonden er wereldwijd grote uitbraken in ziekenhuizen en nam de ernst van de infectie en de mortaliteit toe. Eén van de ruim 400 typen van *C. difficile* bleek geassocieerd met deze uitbraken: PCR ribotype 027 (Hoofdstuk 1). Dit type bleek virulenter dan de ander *C. difficile* types en geassocieerd met de productie van grotere hoeveelheden toxinen en de vorming van meer sporen. Daarnaast bleek dit type resistent tegen de nieuwere generaties fluorochinolonen.

In Nederland werd de eerste grote uitbraak van CDI in 2005 herkend, wat leidde tot de oprichting van een nationaal referentielaboratorium en de start van een surveillance in 13 ziekenhuizen. In Hoofdstuk 2 beschrijven we de moleculaire epidemiologie van *C. difficile* in Nederland tussen 2005 en 2009 (n=2788 monsters). We concludeerden dat *C. difficile* PCR ribotype 027 verantwoordelijk was voor het merendeel van de ernstige gevallen en uitbraken van CDI in 2005 en de eerste helft van 2006. Daarna verminderde het aandeel van type 027. Drie andere types: PCR ribotype 001, 078 en 014 werden toen de belangrijkste verwekkers van CDI in Nederland. De incidentie van CDI in Nederland was stabiel rond de 18 infecties per 10,000 opnames per jaar (Hoofdstuk 2).

Naast de infecties in ziekenhuizen veroorzaakt *C. difficile* ook ziekte buiten het ziekenhuis. In Hoofdstuk 3 evalueren we de beschikbare literatuur hieromtrent. Veel voorkomende risicofactoren, zoals recent antibiotica gebruik of een ziekenhuis

opname, ontbreken in 25% tot 40% van de patiënten die CDI krijgen buiten het ziekenhuis. Dit zorgt er voor dat deze groep moeilijk te herkennen is en dat we niet weten waarom deze patiënten de ziekte krijgen. Omdat *C. difficile* ook gevonden wordt in de darmen van dieren (met name kalveren en biggen), de omgeving (o.a. water en grond) en sommige voedselproducten, worden deze factoren gezien als een mogelijke bron van *C. difficile*. Omdat de absolute aantallen van *C. difficile* laag zijn in voedsel zoals vlees, is infectie hiervandaan minder waarschijnlijk. Neonatale biggen daarentegen kunnen drager zijn van een grote hoeveelheid *C. difficile*, in het bijzonder van type 078. Omdat dit type ook in toenemende mate gezien wordt in humane CDI (nu het derde meest voorkomende type) en 25% van de varkensboeren drager is van de bacterie, lijkt het er op alsof dit type de species-barrière kan doorbreken en er sprake is van zoonotische transmissie. Direct bewijs voor de transmissie van type 078, of een ander type, van dier naar mens is er echter nog niet, daarom concluderen we in dit hoofdstuk dat de incidentie van CDI buiten het ziekenhuis niet wordt bepaald door amplificatie van de bacterie in dieren (Hoofdstuk 3).

Wie worden er ziek?

Een patiënt met CDI is volgens de klassieke beschrijving een persoon op leeftijd met comorbiditeit of recent antibiotica gebruik. Het klassieke risicoprofiel van CDI patiënten is gebaseerd op onderzoek dat tijdens uitbraken is gedaan. In Hoofdstuk 4 onderzoeken wij of het dit risicoprofiel ook geldt in een situatie zonder CDI uitbraak (zoals in Nederland). Wij vergeleken 93 opgenomen CDI patiënten uit het Leids Universitair Medisch Centrum met 76 opgenomen patiënten zonder diarree, en toonden aan dat patiënten met een recente ziekenhuisopname of antibiotica gebruik in de voorgaande drie maanden, een hoger risico op CDI hadden (OR 4,49 en OR 5,41, respectievelijk). Ook een oudere leeftijd (>65 jaar) en een onderliggende ziekte (hoge Charlson comorbidity index) werden vaker gezien bij CDI patiënten, dit verschil was echter niet significant in uni- en multivariate analyse. In tegenstelling tot uitbraken van CDI, was in een endemische setting het gebruik van fluorochuinolonen geen risicofactor voor CDI in onze studie. Dit verschil kan verklaard worden door het ontbreken van CDI door type 027, aangezien dit type geassocieerd wordt met toegenomen resistentie tegen fluorochuinolonen (Hoofdstuk 4).

Hoofdstuk 4 beschrijft de resultaten van een enkel ziekenhuis; vergelijkbare gegevens werden verzameld in 13 Nederlandse ziekenhuizen. In Hoofdstuk 5 gebruiken wij deze data om antibiotica als risicofactor voor CDI in meer detail te

bestuderen. Door 337 CDI patiënten te vergelijken met 337 controles zonder diarree, toonden wij aan dat vrijwel alle antibiotica de kans op CDI vergroten. Daarnaast lieten wij zien dat het risico op CDI hoog is ten tijde van het antibiotica gebruik (Odds ratio 10). Dit risico blijft verhoogd in de eerste maand na het stoppen van antibiotica (Odds ratio 7-10). Hierna neemt het risico op CDI duidelijk af, maar 1 tot 3 maanden na het stoppen van de antibiotica is het risico op CDI nog steeds verhoogd (Odds ratio 2.5) (Hoofdstuk 5).

In Hoofdstuk 6 bestuderen wij patiënten die zich met diarree melden in de huisartsenpraktijk: 12.714 patiënten met diarree en een aanvraag voor microbiologisch onderzoek (niet noodzakelijk voor *C. difficile*) werden getest op de aanwezigheid van *C. difficile*. De ontlasting van 194 patiënten bleek positief (incidentie van 0,67 per 10.000 persoons jaren). Deze incidentie was vergelijkbaar met het aantal patiënten dat een positieve test had voor *Salmonella* spp.. In de huisartsenpopulatie waren *C. difficile* type 002 en 078 veel voorkomend (beide veroorzaakten 11% van alle CDI gevallen). CDI patiënten gebruikten vaker een antibioticum en waren vaker opgenomen in een ziekenhuis in de periode vóór het ontstaan van de diarree, vergeleken met gematchte controles met diarree maar een negatieve test voor *C. difficile*. In ziekenhuizen wordt dit beschreven als het klassieke risicoprofiel van CDI patiënten. Echter, slechts 61% van alle CDI patiënten die zich presenteerden in de huisartsen praktijk, had een dergelijk profiel. Dat betekent dat 39% van de CDI optrad bij mensen zonder bekende risicofactoren, hetgeen een juiste diagnose op basis van de klinische presentatie moeilijk maakt.

Hoofdstuk 6 laat ook zien dat huisartsen op dit moment moeite hebben om CDI te herkennen. Zij detecteren slechts 40% van alle CDI. Het melden van een positieve diagnostische test aan de huisarts resulteerde in 78,7% tot een gerichte behandeling van CDI, maar toch werd nog 4% van de CDI patiënten opgenomen in een ziekenhuis vanwege diarree. Landelijke richtlijnen voor de herkenning van CDI buiten het ziekenhuis adviseren om alle patiënten met diarree of een recente ziekenhuis opname te testen voor *C. difficile*. Gebaseerd op de cijfers van ons onderzoek zou dat 19% zijn van patiënten met diarree die zich bij de huisarts meldden. Bij het volgen van deze richtlijn, zou het aantal gediagnosticeerde CDI patiënten stijgen naar 61%. Een verdere stijging van de detectie kan worden bereikt door een nieuwe predictieregel toe te passen, die wij ontwikkelden in Hoofdstuk 6. Deze regel gebruikt parameters zoals leeftijd, eerder antibiotica gebruik, voorgaande ziekenhuis opname, onderliggende ziekten en de ernst van de symptomen van CDI. Met behulp van deze regel kunnen we, door 44% van de patiënten met diarree te

testen op *C. difficile*, 85% van alle CDI in de huisartsenpraktijk vinden. Hoewel de predictieregel validatie nodig heeft en een kosten-effectiviteitsanalyse, zou deze score een alternatief kunnen zijn voor de huidige richtlijn voor het testen van *C. difficile* (Hoofdstuk 6).

De ernst van de infectie

Patiënten met CDI kunnen milde diarree hebben die zonder een specifieke behandeling verdwijnt, maar soms ontwikkelt de ziekte zich tot een ernstige darminfectie zoals een pseudomembraneuze colitis. Sinds het begin van deze eeuw is niet alleen de incidentie van CDI toegenomen, maar ook de mortaliteit van de aandoening. In Hoofdstuk 7 bestuderen we het beloop van patiënten met CDI in 13 Nederlandse ziekenhuizen (n=1366). Dertien procent van de mensen met CDI overleed binnen 30 dagen. Wij constateerden dat de 30-dagen mortaliteit van CDI patiënten (n=317) 2.5 maal hoger was dan de mortaliteit van vergelijkbare controle patiënten zonder diarree (n=317). Deze hogere mortaliteit onder CDI patiënten was met name te zien in de eerste 30 dagen van de infectie. Een hoge mortaliteit onder CDI patiënten is een aanwijzing dat er een betere behandeling nodig is. Er zijn een aantal nieuwe behandelingen beschikbaar gekomen, maar vrijwel alle patiënten in Nederland worden op dit moment behandeld met metronidazol.

Het is moeilijk om te voorspellen welke patiënten goed reageren op een behandeling voor CDI en welke patiënten een gecompliceerd beloop krijgen (zoals het falen van therapie of overlijden). De Europese vereniging van klinische microbiologie en infectieziekten (ESCMID) stelde een lijst op van mogelijke voorspellers van een ernstige infectie. In Hoofdstuk 8 onderzochten wij of 3 van deze parameters ook daadwerkelijk het falen van therapie konden voorspellen op de dag van de CDI diagnose. In een groep van 1105 deelnemers aan een gerandomiseerde klinische trial van vancomycine versus fidaxomicine, waren koorts (temperatuur boven de 38.5°C), nierfalen (creatinine boven de 133 mmol/L) en leukocytose (leukocyten aantal van meer dan 15*10^9/L) significant geassocieerd met het falen van therapie. Het falen van therapie was gedefinieerd als aanhoudende diarree of de noodzaak voor additionele behandeling van CDI. In een cohort van 104 opgenomen patiënten met sequentieel gemeten laboratorium waarden (±3 dagen rond de diagnose van CDI), lieten wij echter zien dat de creatinine waarden en de leukocyten aantallen van patiënten zeer variabel waren rond de dag van diagnose. Om deze reden concludeerden wij dat leukocytose en nierfalen goede voorspellers kunnen zijn van therapie falen, mits ze gemeten worden op een vast tijdstip. Koorts werd slechts

in 1% van de CDI patiënten gevonden, wat zorgt voor een beperkte waarde als voorspeller.

In Hoofdstuk 9 onderzochten wij de associatie van een bacteriële virulentie marker (binair toxine) met de mortaliteit binnen 30 dagen na de diagnose. In tegenstelling tot de selectie van voorspellers, zoals in Hoofdstuk 8, was het doel van Hoofdstuk 9 om een causaal verband aan te tonen. Binair toxine wordt vaak gevonden in *C. difficile* isolaten die ernstige ziekte geven of een gecompliceerd beloop van de infectie. Er is aangetoond dat dit toxine de adhesie en de kolonisatie van *C. difficile* in het maag-darmkanaal van muizen bevordert. Om binair toxine als oorzaak van een gecompliceerd beloop van CDI te onderzoeken, bestudeerden wij de associatie van binair toxine met de mortaliteit van patiënten met CDI (n=1366 deelnemers). Binair toxine positieve stammen werden gestratificeerd op het niveau van PCR ribotype: type 027 stammen en niet-027 stammen. Type 027 (altijd binair toxine positief) was geassocieerd met een hogere 30-dagen mortaliteit vergeleken met de mortaliteit in patiënten met een binair toxine negatieve stam (22% t.o.v. 11% 30-dagen mortaliteit; HR 2.2, 95% CI 1.2-2.4). Patiënten met een infectie door een binair toxine positieve maar niet-027 stam, hadden slechts een iets verhoogde mortaliteit ten opzichte van binair toxine negatieve stammen (15% t.o.v. 11% 30-dagen mortaliteit; HR 1.5, 95% CI 0.8-2.6). In Hoofdstuk 8 concludeerden wij dat er op dit moment is geen overtuigend bewijs is dat binair toxine de oorzaak is van een hogere 30-dagen mortaliteit in CDI patiënten.

In Hoofdstuk 10 stelden wij een predictiemodel op voor het voorspellen van een gecompliceerd beloop bij patiënten met CDI. Een gecompliceerd beloop werd gedefinieerd als een opname op de Intensive Care afdeling, een colectomie, of dood door CDI binnen 30 dagen na het stellen van de diagnose. We maakten gebruik van een groep van 395 CDI patiënten uit 13 Nederlandse ziekenhuizen en selecteerden een aantal klinische parameters die eenvoudig beschikbaar waren aan het bed van de patiënt ten tijde van de CDI diagnose. Leeftijd, opname in verband met diarree, diagnose op de Intensive Care afdeling, hypotensie en recente abdominale chirurgie bleken voorspellers van een gecompliceerd beloop. Door deze parameters in een predictiemodel samen te voegen, bleek het mogelijk om patiënten te classificeren op basis van hun risico op een gecompliceerd beloop: een hoog risico (39% met een gecompliceerd beloop), matig (16%), laag (5%) of vrijwel geen risico op een gecompliceerd beloop. Dit model werd extern gevalideerd in een klein cohort.

Discussie

In dit proefschrift wordt er een overzicht gegeven van de epidemiologie van *C. difficile* infecties en geven we een aantal aanbevelingen om individuen met een verhoogd risico op het ontwikkelen van CDI te herkennen. We toonden aan dat het klassieke risicoprofiel voor CDI ook geldt buiten uitbraaksituaties (Hoofdstuk 4), we identificeerden nieuwe risicofactoren voor CDI (Hoofdstuk 5) en selecteerden voorspellers voor CDI bij patiënten die zich bij een huisarts presenteren (Hoofdstuk 6). Een tweede doel van dit proefschrift was om factoren te vinden die geassocieerd zijn met een gecompliceerd beloop van CDI. Naast het beschrijven van het huidige beloop van CDI in Nederland (Hoofdstuk 7), beschreven wij de karakteristieken van patiënten met een gecompliceerd beloop van CDI in de daarop volgende hoofdstukken (Hoofdstuk 7, 8, 9, 10). Deze informatie kan bijdragen aan de herkenning van patiënten die een hoog risico hebben op een gecompliceerd beloop van CDI. Omdat de meeste patiënten met CDI in Nederland worden behandeld met metronidazol en niet met vancomycine of fidaxomicine (Hoofdstuk 6), zou het classificeren van patiënten op basis van hun mogelijke uitkomst een rol kunnen spelen in de keuze van het optimale middel (Hoofdstuk 11). Naast het bovenstaande bespreken wij in de discussie een aantal methodologische aspecten van ons onderzoek, plaatsen wij onze bevindingen in de context van recente literatuur en geven wij aanbevelingen voor toekomstig onderzoek.

List of publications

Peer reviewed articles

1. Hensgens MP, Goorhuis A, Notermans DW, van Benthem BH, Kuijper EJ. Decrease of hypervirulent *Clostridium difficile* PCR ribotype 027 in the Netherlands. Euro Surveill. 2009
2. Hensgens MP, Keessen EC, Squire M, Riley TV, Koene MG, de Boer E, Lipman LJ, Kuijper EJ. *Clostridium difficile* infection in the community: a zoonotic disease? Clin Microbiol Infect. 2012
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4. Hensgens MP, Goorhuis A, Dekkers OM, Kuijper EJ. Time-interval of increased risk for *Clostridium difficile* infection after exposure to antibiotics. J Antimicrob Chemother. 2012
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About the author

Marjolein Hensgens (March 1st 1984) was born in Heerlen, the Netherlands. She is the daughter of Wim Hensgens and José van Goor and the elder sister of Juliette. In 2002, she received her certificate of pre-university education (Gymnasium, Bernardinus college, Heerlen). Later that year, Marjolein started her Medical education at Utrecht University. In the first years of this education, Marjolein worked as a nurse in a research institute and trained her younger peers in physical examination. She followed her internships in Utrecht, Rotterdam, Amsterdam and Heerlen, and abroad in Zambia and Nepal. Besides her interest in patient care, Marjolein focused on medical research in the Academic Medical Hospital in Amsterdam and the University Medical Center in Utrecht. To expand her knowledge of medical research, she started a PhD in May 2009 at the department of Medical Microbiology of the Leiden University Medical Center under supervision of prof. dr. Ed J. Kuijper. Together with dr. Olaf M. Dekkers from the department of Clinical Epidemiology, they supervised her research on patients with a *Clostridium difficile* infection. During this PhD, Marjolein gave multiple oral presentations at national and international conferences and followed courses in the field of epidemiology. Currently (2013), Marjolein works as a medical doctor in the department of Internal Medicine at the Meander Medical Center in Amersfoort, where she is trained to become an internal medicine specialist.

Acknowledgements

Het is zo ver: mijn proefschrift is af. Leergierig en met veel enthousiasme heb ik aan de inhoud van dit boekje gewerkt. Bij tijd en wijle werd echter mijn geduld op de proef gesteld. Juist in deze periodes waren de mensen in mijn omgeving een grote steun. Daarom voor jullie allemaal: dank voor de steun en het enorme vertrouwen.

Ed, allereerst dank voor het vertrouwen dat je me gaf door me aan te nemen en de ruimte die je me gaf voor mijn eigen (eigenwijze) inbreng. Maar ook dank voor de momenten dat je liet zien waarom jij de supervisor was en ik de promovendus.

Olaf, zonder jou als co-promotor had mijn proefschrift nooit de epidemiologische diepgang gekregen die het nu heeft. Mijn inzicht in de epidemiologie, maar ook in onderzoek doen in het algemeen, is door jouw didactische vaardigheden en kennis ontzettend toegenomen. Dank dat je 'belangeloos' tijd in mij wilde steken.

C-differs, dank voor de discussies die jullie met mij hebben gevoerd op een kleine 4 vierkante meter. Lief en leed hebben we gedeeld: heerlijk.

Lieve vrienden, dank voor alle leuke etentjes, kopjes koffie en uitgaansavonden die jullie met mij hebben doorgebracht zodat ik even niet over mijn proefschrift hoefde na te denken. Mama, Juul en de families: fijn dat jullie een grenzeloos vertrouwen in mij hebben, zelfs als ik denk dat dat niet gegrond is. Thijs, een fijn thuis en jouw lieve zorgen maakten de afgelopen jaren een feest. Dat dat nog maar lang zo mag blijven.

Dit proefschrift was nooit tot stand gekomen zonder de medewerking van alle mensen met een *Clostridium difficile* infectie die meewerkten aan mijn studies. Vele vragenlijsten vulden zij in, om zo een grote hoeveelheid data te creëren waarmee epidemiologisch onderzoek gedaan kon worden. Dank ben ik ook verschuldigd aan de laboratoria die deze patiënten testten op *C. difficile* en vervolgens bij ons aanmeldden; en aan het referentie laboratorium dat zorgde voor een juiste typering van alle isolaten.