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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