CHAPTER 14

SUMMARY OF RESULTS AND GENERAL DISCUSSION

SUMMARY OF MAIN FINDINGS

THE INTESTINAL MICROBIOTA DISRUPTED

In 2013-2014, a large outbreak of *Clostridium difficile* ribotype 027 occurred at the VU University medical center (VUmc) in Amsterdam, The Netherlands. An absolute nightmare for the hospital, but for me also an opportunity to start this research project. At that time, an important question was whether (changes in) hospital-associated factors may have increased the risk of developing *C. difficile* infection (CDI), and therefore contributed to the hospital wide spread of the outbreak strain. Additional questions were whether the outbreak strain was associated with a higher recurrence risk, and if a recurrence or complicated course of CDI could be predicted. Furthermore, we wanted to provide insight into the financial burden that the outbreak brought upon this hospital. These questions are addressed in Part I and II of this thesis, and will be summarized in the following paragraphs.

Part I: An outbreak of *Clostridium difficile* infection

During the outbreak, use of antibiotics, recent admission to VUmc, a longer duration of hospital stay, and prior admission to the intensive care unit (ICU) were associated with the development of CDI **(Chapter 2)**. That 95% of CDI patients had used antibiotics in the three months before diagnosis, implies that antibiotics are almost an essential prerequisite to develop CDI, even during an outbreak. Almost half of patients diagnosed with CDI ribotype 027, but only one-sixth of the non-CDI controls, and one tenth of the non-027 CDI patients had been admitted to the ICU prior to CDI diagnosis. These differences point to the ICU as important contributing factor. For those patients who had been admitted to the ICU prior to CDI diagnosis, the use of selective decontamination of the digestive tract (SDD: intestinal and oropharyngeal application of an antibiotic/antifungal cocktail, which is given by protocol to all patients admitted to the ICU with an expected length of stay of more than 48 hours), and a prolonged length of ICU stay were both associated with the development of

CDI. It has been shown that a larger amount of antibiotics, more different classes of antibiotics, and a longer duration of antibiotic therapy are all associated with an increased risk of CDI.[1,2] Therefore, although the effect of SDD and a prolonged length of ICU stay could not be disentangled in our study, the increased risk for the development of CDI after the use of SDD appears a biologically plausible finding. Our findings suggest that the ICU population demands additional resources and attention from an infection prevention perspective to control spread of *C. difficile* in future outbreaks. This may also include restriction of SDD use.

A major challenge in the management of CDI is the high recurrence rate, which ranges from 20% after a first episode, to 65% after multiple episodes.[3] The recurrence rate of CDI has increased since early 2000, coincident with the emergence of *C. difficile* ribotype 027. This suggests a possible link between ribotype 027, and the development of recurrent disease.[4] During the outbreak, 32% of patients with CDI due to ribotype 027 developed a recurrence within 30 days after completion of initial CDI treatment, compared to 25% in patients with CDI due to other ribotypes. Although this difference is not large, an additive role for the more virulent ribotype 027 could not be ruled out. However, in our cohort, age and duration of hospitalization were the main risk factors for recurrent CDI. This points to host and demographic factors as more important predictors for recurrent CDI than strain type **(Chapter 3)**.

The financial burden of the outbreak was substantial, we estimated it to be at least €1,222,376 **(Chapter 4)**. Closure of beds because of contact isolation requirements and prolonged length of hospital stay of CDI patients especially, contributed to the increased costs. The high costs associated with this outbreak justify the use of additional resources for CDI prevention and infection control.

Part II: Prediction tools for complications after *Clostridium difficile* infection

Estimating the risk of a complicated course of CDI or recurrent disease might help doctors guide treatment. In the last two decades, multiple clinical scoring systems to predict a complicated course or recurrence have been developed.[5-7] However, due to lack of external validation, the study design, or the limited number of patients on which they are based, none has gained widespread clinical acceptance.

Using the outbreak cohort, complemented with patients that had been diagnosed with CDI due to other ribotypes during the same period, we provide external validation of three different prediction tools for patients at risk of a complicated course of CDI in **Chapter 5.**The performance of all three prediction tools was very poor when applied to our total cohort. However, when patients diagnosed with CDI due to the outbreak strain were excluded, one prediction model[8] performed much better. This prediction model, which includes age, department of diagnosis, recent abdominal surgery, hypotension, and diarrhea as reason for admission as predictors, can therefore be of additional value for clinicians in deciding which CDI patients to monitor more closely.

Better identification of patients at risk for recurrent CDI could improve treatment strategy, for instance by considering Fecal Microbiota Transplantation (FMT), fidaxomicin (narrow spectrum, non-absorbed, macrolide antibiotic), or bezlotoxumab (monoclonal antibody), since these treatments are associated with a lower recurrence risk.[9-11] Previous studies indicate that the composition of the intestinal microbiota is an important determinant of recurrent CDI.[12,13] Differences in the composition of the gut microbiota result in differences in volatile organic compounds (VOC) composition of fecal samples.[14] Therefore, VOC analysis has the potential to provide a direct reflection of the physiological status of the gut microbiota. In a small pilot study, the VOC profile of CDI patients who developed a recurrence differed from the profile of CDI patients who did not develop a recurrence **(Chapter 6)**. This suggests that VOC

profiling should be further evaluated as a possible tool to predict recurrent CDI. However, first, reproduction in a larger study, and external validation are required.

THE INTESTINAL MICROBIOTA RESTORED

In 2013, the efficacy of FMT for recurrent CDI was confirmed in a randomized controlled trial, with a cure rate of 85%.[9] We felt that, after experiencing such a large outbreak, we had to be able to offer this effective treatment to our patients. Using ready-to-use donorfeces from OpenBiome (a nonprofit organization that maintains a stool bank in Boston, USA), we started to treat patients with multiple recurrences with FMT. Almost three years later, the Netherlands Donor Feces Bank (NDFB) has been developed to optimize FMT treatment for CDI in The Netherlands. At VUmc we have just finished a pilot study to evaluate possible treatment of patients with Irritable Bowel Syndrome (IBS) with FMT. Our experiences regarding FMT are discussed in the third and fourth part of this thesis.

Part III: Fecal Microbiota Transplantation for *Clostridium difficile* infection

Treatment with FMT has received increasing interest. However, more (long-term) follow-up data are needed to capture the safety profile of FMT. Follow-up of almost 40 patients who were treated with FMT by duodenal tube for recurrent CDI at the Academic Medical Center in Amsterdam, reveals no long-term side effects (e.g. infectious complications, auto-immune disease, obesity, diabetes) after FMT treatment **(Chapter 7)**. However, 13% of the treated patients experienced regurgitation or vomiting after FMT. This implies that particularly in patients with increased risk for aspiration, FMT by colonoscopy or enema is indicated (discussed later in 'clinical implications'). Furthermore, our study shows that a first post-FMT recurrence can be successfully treated with antibiotic therapy, without the need of repeat FMT.

To date, FMT is mainly used as rescue therapy after multiple recurrences. However, originally (1958), FMT was conceived as a curative treatment modality for severe, refractory CDI with ongoing pseudomembranous colitis.[15] Current treatment guidelines suggest oral vancomycin (or, in case of ileus, rectal vancomycin) with or without metronidazole intravenously for severe, or complicated disease.[16] When antibiotic treatment is not sufficient, abdominal surgery is indicated.[17] In **Chapter 8**, we review, and discuss literature about the curative use of FMT in severe or complicated CDI. The existing case data suggest that FMT, with or without additional antibiotic CDI treatment, seems to be a promising curative treatment option for severe and/or complicated CDI, which should be considered before proceeding to surgery.

In **Chapter 9**, we describe a 14 year old child with Down syndrome treated with FMT for recurrent CDI. Our findings indicate that also children with Down syndrome, that is characterized by an alternate immune system, may well tolerate and benefit from FMT, even in severe immunocompromised state due to comorbid malignancy. Detailed microbiota analysis showed that the patient developed a unique microbiota profile after FMT which was very stable over time.

Part IV: Fecal Microbiota Transplantation: other indications and the development of the Netherlands Donor Feces Bank

To date, recurrent or refractory CDI are the only indications for FMT. In **Chapter 10**, we discuss the potential of FMT in the treatment of other diseases associated with alterations of the gut microbiota, including inflammatory bowel disease (IBD), IBS, metabolic syndrome, graft versus host disease, and eradication of fecal carriage of multidrug resistant organisms. Additionally we discuss modulation of behavior by infusion of human donorfeces in mice. Data of three randomized controlled trials show that FMT induces remission in approximately 24-30% of patients with ulcerative colitis, compared with 5% (water) to 20% (autologous feces) in placebo treated

patients.[18-20] Additionally, in a small randomized controlled trial, it has been shown that in patients with metabolic syndrome FMT may increase insulin sensitivity.[21] For the other indications, publications about FMT are limited to case-reports and case-series. However, the results are promising.[22-25]

That FMT appears as a promising therapy for post-infectious and antibiotic-induced IBS is demonstrated in an open label observational pilot study of which we describe the results in **Chapter 11**. In this pilot study (N = 10), treatment with a single FMT by duodenal tube, did result in clinical response in 50% of the participants. Especially severity of abdominal pain, satisfaction about bowel habit, and influence of IBS symptoms on daily life improved after FMT. Importantly, microbiota analysis shows that the microbiota composition of responders shifts to that of their corresponding donors. Non-responders initially also do, however, eight weeks after FMT their microbiota composition shifts back towards baseline. This finding suggests that the effect of FMT in IBS may be associated with sustained engraftment of donor microbiota. However, randomized placebo controlled trials are needed to determine treatment effect, and to provide more reliable analyses of the association between clinical IBS symptoms and microbiota composition.

In **Chapter 12** we describe a patient with refractory celiac disease type II (RCD II) who received FMT as treatment for recurrent CDI. This patient had suffered for over 10 years from villous atrophy, despite a gluten free diet and additional therapy. Remarkably, duodenal biopsies obtained six months after FMT showed complete recovery of the villous atrophy. It is tempting to speculate that altering the composition of the microbiota could improve the clinical and histological consequences of RCD II. Therefore, we think that such novel treatment modality, should be further investigated.

In 2015, the Netherlands Donor Feces Bank (www.ndfb.nl) was founded with the primary aim to provide ready to use, high-quality donorfeces solutions for treatment of recurrent CDI in The Netherlands. **Chapter 13** addresses the current donor recruitment and screening, preparation of the fecal suspensions, legislation of FMT, transport of the fecal suspensions to different hospitals, and the experiences and follow-up of patients treated with donorfeces of the NDFB. Of the 165 volunteers who registered as a potential feces donor, only four volunteers (2.4%) were eligible at the end of the screening process (including a quarantine period of two months). Importantly, 60% of volunteers who passed the first set of clinical, serological, and stool screening, did not pass the second set of screening tests after a two month quarantine period. These numbers highlight the importance of a quarantine period, and the necessity of central stool banks. In May 2016, the first FMT with a donorfeces suspension of the NDFB was performed. Up to September 2017, 60 NDFB feces suspensions have been distributed to 20 different hospitals throughout the Netherlands.

IMPLICATIONS FOR CLINICAL PRACTICE

Clostridium difficile infection

The outbreak at VUmc involved nearly 80 patients infected with *C. difficile* ribotype 027 before coming to an end. To control the outbreak several infection control

measures were implemented: reinforcement of infection control (use of aprons and gloves, appropriated hand hygiene, contact isolation of every patient with diarrhea in single rooms, and the introduction of hydrogen peroxide as disinfectant), extra cleaning, optimization of CDI diagnosis (every patient with diarrhea was tested twice for toxin-producing *C. difficile* even when the diarrhea was likely attributable to an underlying condition or therapy), optimization of CDI treatment (most patients were treated with a combination of metronidazole and vancomycin), and antibiotic stewardship. After the implementation of these infection control measures, the incidence of CDI due to ribotype 027 decreased drastically. At present, over three years after the outbreak, the incidence of CDI in our hospital remains very low with an incidence of 4.5 patients with CDI per 10,000 patient-days from January to April 2017 (Figure 1). In this period, no cases of CDI due to ribotype 027 occurred.

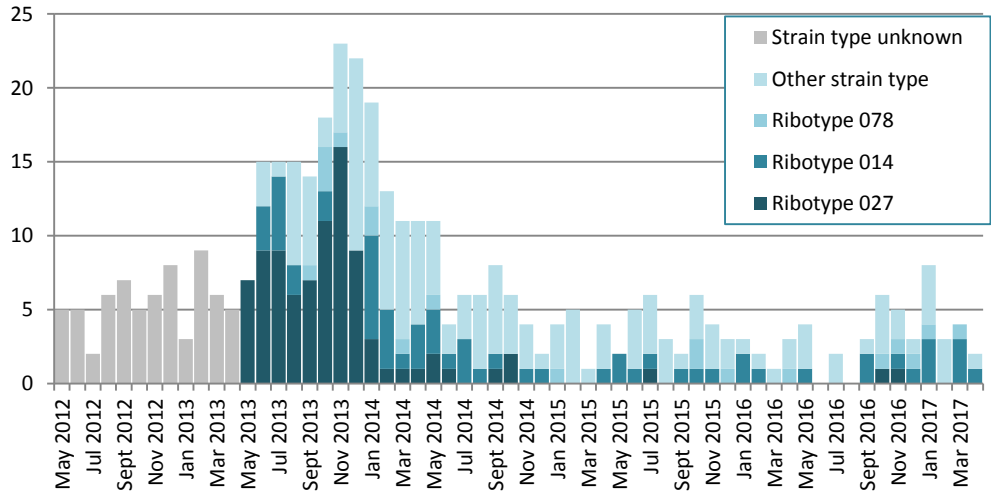

Figure 1. Monthly incidence of *Clostridium difficile* infection from May 2012 to April 2017. Data provided by drs. A.M. Kaiser, Department of Medical Microbiology and Infection Control VUmc

Whole genome sequencing showed that the outbreak was likely caused by a single strain of *C. difficile* ribotype 027*,* which underscores the transmissibility of this hypervirulent strain. However, as shown in Figure 1, other strains of *C. difficile* remained endemic. The high rate of transmission of *C. difficile* ribotype 027, and the low rate of transmission of other strains of *C. difficile* suggests that in patients infected with non-hypervirulent strains of *C. difficile* strict contact precautions may not be necessary. This suggestion is recently substantiated by a Swiss study: after discontinuing contact precautions for patients with CDI (except for patients diagnosed with hypervirulent strains 027 or 078, or patients with stool incontinence), over a 10-year period, the rate of transmission of toxigenic, predominantly non-hypervirulent *C. difficile* was still low and no outbreaks occurred.[26]

Patients at risk for (recurrent) CDI might benefit from a different treatment strategy at an earlier stage. Furthermore, a more strict isolation policy could be appropriate for this group of patients. In the first two parts of this thesis, we have learned that especially older patients, who receive a lot of antibiotics and have a prolonged length of hospital stay, are the most at risk for initial or recurrent infection with *C. difficile.* Additionally, patients admitted to the ICU represent a highly vulnerable population due to extensive use of antibiotics (including SDD), severe underlying disease, exposure to invasive procedures, and frequent contacts with healthcare workers that increase potential exposure to spores of *C. difficile*. Although SDD has been proven to prevent severe infections and to reduce mortality in critically ill patients[27], we should recognize its collateral damage to the gut microbiota.[28] Since the imbalance in the gut microbiota plays a key role in the pathophysiology of CDI, restricting the use of SDD exclusively to patients who really need it, may prevent further spread of *C. difficile* during an outbreak.

To avoid another outbreak with a hypervirulent strain of *C. difficile*, recommendations on infection control measures should be followed at the earliest possible stage, with

extra attention for the ICU department since they receive and discharge our most severely ill patients to all the different wards of the hospital.

Fecal Microbiota Transplantation

The introduction of the NDFB has made it possible to treat every patient with recurrent CDI in their local hospital. This greatly simplifies the logistics of FMT, and also contributes to its implementation in clinical practice. Additionally, the availability of ready-to-use donorfeces makes it possible to perform urgent FMTs for severe or complicated CDI. Last year, we have successfully treated a patient who suffered from severe pseudomembranous colitis with urgent FMT, followed by fidaxomicin, a second FMT, and another course of fidaxomicin. This new treatment strategy for patients with severe or complicated CDI, who do not respond to conventional antibiotic treatment, should be considered in all patients before proceeding to colectomy because of severe colitis.[29]

Donorfeces is usually administered through a duodenal or gastric tube, colonoscopy, or enema. In general, FMT is considered to be safe. However, septic shock with decompensated toxic megacolon, aspiration during sedation for colonoscopy, fatal aspiration, and one case of cecal perforation necessitating colectomy have been described.[30-34] In addition, we have reported five patients who experienced regurgitation or vomiting of donorfeces after FMT by duodenal tube (Chapter 8). Our report implicates that patients with swallowing disorders should be excluded from FMT by upper gastrointestinal delivery due to this risk of regurgitation or vomiting with subsequent aspiration of donorfeces. On the other hand, patients with a severe inflamed colon should be excluded from FMT by lower gastrointestinal delivery due to the risk of perforation. Since all delivery methods have their advantages and disadvantages, the ideal route of delivery should be assessed individually for each patient.

Initially, patients were treated with fecal suspensions up to 500cc.[9] However, the duodenum has only a limited distension capability, and with regard to nutrition it is known that duodenal feeding should always be carried out continuously by pump, and not by boluses.[35] Therefore, it is very likely that the amount of 500cc donorfeces suspension contributed to the complications described in Chapter 8. Since it has been suggested that, to be effective, the required amount of donorfeces should be 50 gram[36], it was possible to lower the volume of donorfeces suspension to a maximum of 200cc. To avoid regurgitation or vomiting of donorfeces when administered through a duodenal tube a maximum of 200cc of donorfeces suspension should be administered very slowly.

Besides the treatment of CDI, there have been a few randomized controlled trials (three for ulcerative colitis[18-20], one for metabolic syndrome[21]), and numerous case-reports and case-series reporting success of FMT for various other intestinal, but also extra-intestinal diseases (discussed in Chapter 10). With clinical success stories, and cure rates up to 90% in case-series, results are promising. In our open label pilot study, where we treated 10 patients with post-infectious or antibiotic-induced IBS with FMT through a duodenal tube, FMT was considered effective in 50% of the participants. Although this pilot study was too small to draw firm conclusions, FMT shows promise as treatment for this subset of IBS patients. However, it is important to note that initial cure rates of FMT for ulcerative colitis in case series, were not confirmed in randomized controlled trials. Therefore, results from case-series, including our pilot study, should be interpreted with caution.

FUTURE PERSPECTIVES

Due to the ageing population and increased antibiotic resistance, it is expected that the number of patients with CDI will further increase worldwide. As mentioned

throughout this whole thesis, we do know that a disrupted gut microbiota is at the core of CDI pathogenesis.[12] However, while the general importance of the gut microbiota in CDI development is well established, little is known about its prognostic implications. Additionally, we are unsure which microbes are responsible for protection or susceptibility of CDI.[37] Further detailed information about the role of the gut microbiota in prevention of (recurrent) CDI is essential for the development of prophylaxis and future therapies for (recurrent) CDI.

In a large subset of patients (approximately 75%), a first episode of CDI can successfully be treated with antibiotics. However, the other 25% develops a recurrent infection, which is a major challenge in the management of CDI. It is generally assumed that the inability of the gut microbiota to re-establish colonization resistance after initial CDI adds to recurrence risk.[37,38] Therefore, it appears paradoxical that current standard treatment for CDI involves the administration of antibiotics, since especially antibiotics have profound (negative) effects on the gut microbiota.[37] Also oral vancomycin, which is frequently used for the treatment of CDI, influences the balance of the intestinal microbiota.[39] To date, restoration of the healthy gut microbiota by FMT is mostly used as a rescue therapy for patients suffering from a second recurrence or more. However, since patients with recurrent CDI are subject to complex and expensive care, and since the social impact is high due to prolonged isolation and uncontrollable diarrhea, why treating a recurrence with FMT instead of preventing it with FMT? Studies that look into the stratification of patients based on their recurrence risk to identify patients that will benefit from early FMT are warranted.

Since the introduction of FMT as treatment for recurrent CDI, its potential for treatment of other diseases with a disrupted gut microbiota has been discussed. However, randomized controlled trials in ulcerative colitis have shown that FMT is not always a ‘one size fits all’ treatment.[18] Although our experiences with FMT have

improved over the last years, there are still some important unresolved questions, including: Is the efficacy of FMT donor or patient dependent? Is bowel preparation required prior to FMT? Do patients benefit from multiple FMTs? Is a higher diversity of donorfeces associated with increased efficacy? What delivery route is most effective? Should we combine FMT with changes in lifestyle or diet? And how do we increase engraftment of donor microbiota into the recipient?

To answer these questions, we first need a solid basic knowledge of the variations of the gut microbiota in the average, healthy population. Although the body of evidence in this field has increased exponentially over the past years, there are still some important issues. First, due to the variability of microbial composition in each individual, a large set of samples needs to be collected with minimal variability in the methods of collection, DNA extraction, or microbiota analysis. Second, host, but also environmental factors such as diet, use of medication, age, place of residence, and underlying comorbidity, independently influence the composition of the gut microbiota.[40-42] In addition, strong association of stool consistency with microbiota composition has recently been observed.[43,44] This implies that in studies of the gut microbiota, information about the variables mentioned above should be included. Third, the majority of microbiota data comes from US, and Europe, with very few studies from Asia or Africa, which biases our view of the 'normal' gut microbiota. Fourth, microbiota composition differs in different parts of the digestive tract.[45-47] It should be realized that fecal samples do not necessarily represent the situation in the proximal colon, and even less that in the small intestine. Fifth, although numerous diseases have been associated with an altered gut microbiota, we still do not know whether these changes are cause or consequence of the disease. At the time of diagnosis, the alterations may just reflect continuous inflammation, or alterations in host defense. Nonetheless, in mice models, causality seems to be proven in most

cases.[48-55] Finally, the majority of data include bacterial composition; less is known about the role of the other gut microbes, such as yeasts, parasites or viruses.

Microbiota analysis of extensively phenotyped cohorts like that of the Human Microbiome Project[56], the Dutch LifeLines-DEEP study[40], and the Belgian Flemish Gut Flora Project[57], has revealed the extent of variation within the 'normal' gut microbiota. Combination of two cohorts mentioned above[40,57], complemented with global data sets, identified a core microbiota (i.e., shared by 95% of samples) consisting of 14 genera.[57] This global identification of a human core microbiota has enlarged our knowledge about a 'healthy' gut microbiota, but even in this large cohort with data from almost 4000 individuals, the total gut diversity is not yet fully covered. Additionally, we are still unsure whether the microbiota's function may be more important than any individual member of the gut microbiota community.

Taken together, a healthy or unhealthy gut microbiota is hard to define at best. However, we do know that the gut microbiota represents an important modulator of human health that can be altered by diet, medicines, or gut modulating therapies, in contrast to other important contributing factors like age and genetics. At the time of writing, we are still at the beginning of understanding the microbiota-host interaction and its consequences. However, research in the composition, function, and diversity of the gut microbiota evolves rapidly, and currently over 100 clinical trials are underway looking into the possible effects of FMT in different diseases. It is expected that this research will help to answer questions about the function of the intestinal microbiota, its key-stone species, and the working mechanism of FMT, which will sooner or later result in standardized (and more appealing than donorfeces) 'second generation probiotics'. Most important, once we understand the role of the gut microbiota in health and disease, it will open new treatment possibilities for a wide range of diseases.

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