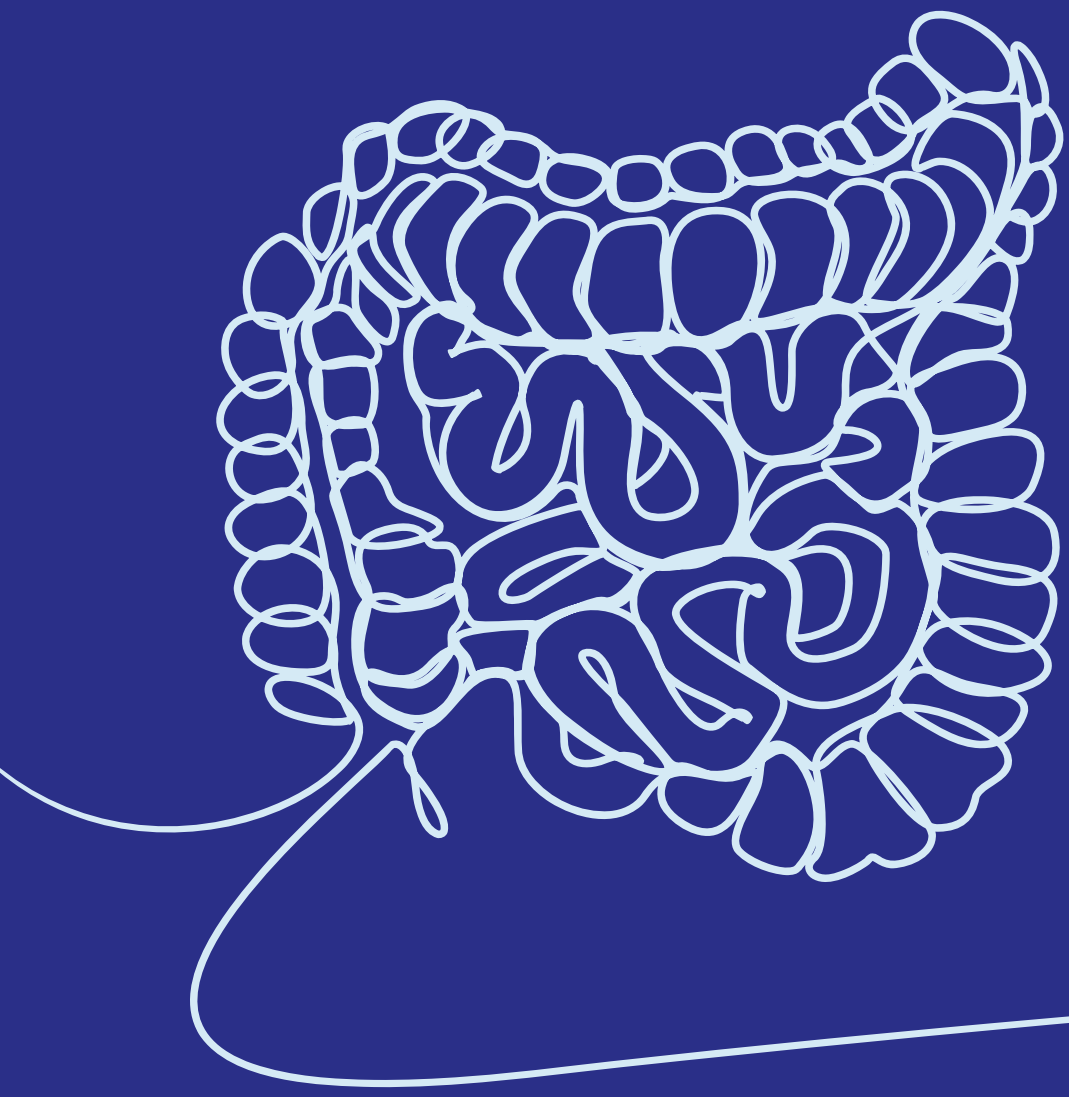
Towards understanding Clostridioides difficile colonization

Crobach, M.J.T.

CHAPTER 9

Summary and general discussion



Before putting the findings from this thesis in perspective, we will summarize our main findings.

Main Findings

In CHAPTER 2, an extensive review of the literature on diverse aspects of *C. difficile* colonization (CDC) is provided. As there are no uniform criteria for CDC, we started our review by clearly defining CDC, also to be able to discern colonization from infection (Table 1). As patients with continuous or prolonged colonization may very well differ from patients who are found to carry *C. difficile* only at one point in time, we further subdivided colonization in transient and persistent colonization (Table 1).

Table 1. Definitions for *C. difficile* infection (CDI) and *C. difficile* colonization (CDC)

C. difficile infection (CDI)	presence *of C. difficile* toxin (ideally) or a toxigenic strain type and clinical manifestations of CDI[1]
C. difficile colonization (CDC)	detection of the organism in the absence of CDI symptoms
transient	*C. difficile* detected at one point in time
persistent	*C. difficile* detected at several points in time

[1] Clinical presentations compatible with *Clostridioides difficile* infection include diarrhea (defined as Bristol stool chart types 5 to 7 plus a stool frequency of three stools in 24 or fewer consecutive hours, or more frequently than is normal for the individual), ileus (defined as signs of severely disturbed bowel function, such as vomiting and absence of stool with radiological signs of bowel distention), and toxic megacolon (defined as radiological signs of distention of the colon, usually to ≥10 cm in diameter, and signs of a severe systemic inflammatory response)

Not only definitions, but also the diagnostic methods to establish CDC are not clearly described in the literature. When considering how to test for CDC, it is important to first assess how CDI should be diagnosed, as incorrect use of laboratory assays for CDI detection can cause overdiagnosis of CDI and underestimation of CDC. In CHAPTER 3, updated recommendations for diagnosing CDI are provided. After publication of the first ESCMID recommendations for diagnosing *C. difficile* infections (1), NAATs had become commercially available and were more and more used as the test of choice, often as stand-alone assay. To better define the role of NAATs in CDI diagnosis, there was a need for updated recommendations. These recommendations were based on a literature review and meta-analysis of studies evaluating test performances of commercial CDI assays. Results showed that although NAATs are highly specific, their positive predictive values (PPV) will be too low at low pre-test probability (as will be the case in stool samples submitted for CDI testing). We therefore recommended against the use of NAATs for stand-alone use. In

line with the previous ESCMID guidelines, we recommended to combine two assays in an algorithm to decrease the percentage of false-positive results. The algorithm should start with an assay with a high negative predictive value (NPV) (i.e. a highly sensitive test) that reliably classifies samples with a negative test result as non-CDI. All samples with a first positive test result will be tested with a second assay with a high PPV (i.e. a highly specific test) that reliably classifies samples with a positive test as CDI. Both GDH EIA and NAAT are suitable as the first assay in the algorithm as they are sensitive assays. The second assay could preferably be a Toxin A/B EIA as these are among the most specific assays and have the additional benefit of detecting free toxins (thought to correspond to clinical disease). (2) Some pitfalls in CDI diagnosis were not addressed in the ESCMID guidelines. These include for example the awareness that PCR RT023 produces colourless colonies on the chromogenic agar ChromID and the possibility of CDI due to *C. difficile* strains that are positive for binary toxin only (and hence will not be detected with most commercial NAATs). (3) These exceptions once again illustrate that interpretation of CDI test results may not always be straightforward.

Although algorithmic testing offers the most accurate CDI testing method, the drawback is that samples need to be tested by multiple assays which may delay diagnosis. Therefore, in CHAPTER 4 we determined whether quantitative results of a NAAT (when used as a first assay in a two-step algorithm) could predict the result of the subsequent Toxin A/B EIA assay. In NAAT, lower cycle quantification (*Cq*) values correspond to higher bacterial counts, and we hypothesized that lower *Cq* values would also be predictive of the presence of toxins. For the analysis, we used a collection of samples submitted for CDI testing to two hospital laboratories (n=2669 and n=1718, respectively). Moreover, we included samples from patients with asymptomatic CDC on hospital admission (samples were derived from the CDD study, see CHAPTER 6). We found significantly lower *Cq* values in stool samples that tested positive for toxins. Using receiver operating characteristic curve analysis, we demonstrated that with the optimal *Cq* cutoff values, prediction of the toxin A/B EIA results was accurate for 78.9% and 80.5% of samples in hospital 1 and 2, respectively. We concluded that *Cq* values can indeed serve as predictors of toxin status, and might possibly aid in establishing a preliminary diagnosis. Yet, due to the suboptimal correlation between the two tests, additional toxin testing is still needed. Interestingly, comparable *Cq* levels were found in CDC patients and diarrheal patients testing negative for toxins, suggesting that these groups are indeed only colonized by *C. difficile* and that the latter group represented patients colonized by *C. difficile* with diarrhea due to another cause

than CDI. Our results also show that reliance on NAAT as stand-alone test may lead to overdiagnosis of CDI and underestimation of CDC.

In CHAPTER 2, we clarified which assays that are traditionally used for diagnosing CDI can also be applied to detect CDC. A specific concern when assessing CDC is that bacterial counts may be lower than in CDI, and therefore the most sensitive methods – like culture- should be used. Also, it is very important to confirm the absence of symptoms suggestive of CDI to prevent confusion between these two conditions. Further in this discussion, we will elaborate more on the risks of confusion between CDC and CDI depending on test methodology and the consequences hereof.

With the realization that there are patients who are colonized with *C. difficile*, but do not demonstrate symptoms, a question arose: which mechanisms allow for colonization but protect against symptomatic disease? The immune system is thought to play an important role. Antibodies directed to the proteins on the surface of *C. difficile* may protect against colonization. (4-6) On the other hand, antibodies directed to the toxins of *C. difficile* may protect against disease by neutralization of toxins. (7) By limiting the detrimental effects of the toxins on the gut epithelium, these latter antibodies may also add in a faster restoration of colonization resistance. (8) Besides the immune system, the gut microbiota seems to play an important role in the susceptibility for *C. difficile* colonization and infection. At the start of this thesis, only a few small studies had characterized the microbiota of patients with asymptomatic CDC. (9, 10) These studies and mouse study results suggested that colonization could be established in microbiota with a decreased species richness and decreased microbial diversity. The presence of specific bacterial taxa was thought to protect from progression to CDI. The paucity of available data on this topic made us decide to characterize the microbiota of colonized patients. In addition, we wanted to compare the microbiota of colonized patients with that of non-colonized patients and symptomatic CDI patients, to be able to identify which microbiota composition is associated with resistance or susceptibility to CDC and CDI. For this analysis, we used a subset of samples obtained during the CDD study (see CHAPTER 6): 41 samples from colonized patients and 43 samples from controls. The third group consisted of 41 samples of symptomatic CDI patients obtained for sentinel surveillance purposes. Gut microbiota composition in these three groups was determined using 16S rRNA gene amplicon sequencing of which the results are described in CHAPTER 5 of this thesis. Bacterial diversity was decreased both in CDC and CDI patients, but the microbiota composition in CDC patients differed from that in CDI patients. The genus *Veillonella* was more abundant in CDI patients, and

also found to be positively associated with *C. difficile* in colonized patients. *Veillonella* may therefore indicate susceptibility to colonization and infection by *C. difficile*. *Eubacterium hallii* and *Fusicatenibacter* were more abundant in control patients than in colonized patients. Also, *Fusicatenibacter* was negatively associated with *C. difficile* in CDI patients. Thus, *Eubacterium hallii* and *Fusicatenibacter* may indicate resistance against CDC and subsequent infection. In CHAPTER 5, we also speculated on the underlying mechanisms for these findings, i.e. the influence of these microbiota members on bile acid metabolism. Although we could not demonstrate causality in this study, the identification of these specific genera may be useful for future studies.

Another part of this thesis focuses on the implications of CDC. Prior to the start of my research, a number of studies had shown that a considerable part of new CDI cases could not be explained by transmission from other known CDI cases. (11, 12) In one study 29% of new CDI cases were associated with asymptomatic *C. difficile* carriers, although this may have been an overestimation as the typing method in this study (MLVA) may have been not discriminative enough. (13) However, the interest in other possible *C. difficile* reservoirs and specifically *C. difficile* colonized patients as the source for new CDI cases grew. Patients who are already colonized at hospital admission gained special interest as it was already known that these patients can introduce *C. difficile* into the hospital and transmit it to other patients. (14) In studies, rates of asymptomatic CDC among patients at hospital admission were reported to range from 3% to 21% (CHAPTER 2). Risk factors for CDC at hospital admission that were identified in these studies included recent hospitalization, chronic dialysis, corticosteroid/ immunosuppressant use, gastric acid suppressant use and antibodies against toxin B. (15-17) Apart from being a potential source for onwards transmission, several studies also pointed towards a higher risk for patients colonized by toxigenic strains to subsequently develop CDI. (18) In order to estimate the prevalence of CDC in the Netherlands and study onwards transmission from asymptomatic carriers, we designed the CDD study (*Clostridioides difficile* colonization study, ***C****lostridioides* ***d****ifficile* **d**ragerschap studie in Dutch). We thought that this study would provide valuable information on CDC prevalence and *C. difficile* transmission in a setting where CDI is endemic with a stable CDI incidence and low prevalence of 'hypervirulent' strains. Result from the CDD study are described in CHAPTER 6 of this thesis. In this multicentre study, we were able to screen 2211 patients in 4 hospitals within 72hrs of hospital admission. We found that CDC was present in 4.9% (108/2211) of admissions, while colonization with toxigenic *C. difficile* strains (tCDC) was present in 3.1% (68/2211) of admissions. To evaluate the consequences of CDC, patient were followed up for progression to CDI. None of the colonized patients

developed CDI during admission or one-year follow-up (one-year follow-up available for 38 colonized patients). In addition, isolates from patients colonized by toxigenic strains were compared with isolates from patients that were diagnosed with CDI during the study period. Core genome MLST (cgMLST) demonstrated no definite transmission from tCDC patients to CDI cases. Only one probable onwards transmission event from a CDC patient was detected as two patients with genetically identical strains shared a ward before the first patient was found to be colonized and the other was diagnosed with CDI. Moreover, the only transmission between multiple symptomatic patients detected by cgMLST was a PCR RT826 cluster that was already detected via sentinel surveillance. This unusual outbreak is described as an outbreak report in CHAPTER 7 of this thesis. The outbreak involved five patients, of whom two had recurrent disease. Clinical case investigations and microbiological analyses including whole genome sequencing showed that all episodes were due to clonal spread of a unique ribotype that was never recognized before. The newly identified ribotype was assigned PCR RT826. This new ribotype resembles the 'hypervirulent' PCR RT078, belongs to clade 5 and carries all three toxin genes (*tcdA*, *tcdB* and binary toxin genes). No definitive source for this newly identified strain could be demonstrated. Yet, the absence of this ribotype in international databases of *C. difficile* strains found in humans and the observation that most ribotypes of clade 5 can also be found in animals (19), made us speculate that this newly identified ribotype might have derived from an animal source. Either the index patient, an undiagnosed CDI patient or an asymptomatic carrier might have introduced this strain into the ward, where it was further transmitted among susceptible patients. Since this outbreak, *C. difficile* PCR RT826 has neither been found in sentinel surveillance samples or in outbreak studies in the Netherlands, indicating that rapid recognition and early implementation of additional infection control measures are important to prevent further spread within the hospital and to other healthcare facilities.

As discussed in CHAPTER 6, screening for CDC on admission like we performed in the CDD study was time-consuming and burdensome but did not detect patients that contributed to *C. difficile* epidemiology by progression to symptomatic CDI. At most one patient contributed to *C. difficile* epidemiology by onwards transmission. Therefore, we concluded that screening for CDC at hospital admission is of little value in an endemic setting with low prevalence of 'hypervirulent' ribotypes.

C. difficile transmission extends beyond the hospital and CDI is increasingly reported in the community (20, 21).

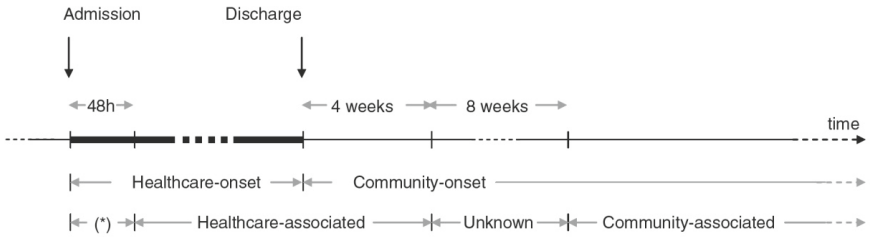


Figure 1. Definitions for community-associated and healthcare-associated *Clostridioides difficile* infection (CDI).
Adopted from Kinross *et al.* (22)

In CHAPTER 8, we used data obtained for our national sentinel CDI surveillance program from 2012 to 2018 to analyse characteristics of CDI patients with community-onset of symptoms (CO-CDI) and subsequent hospitalization. CO-CDI can be community-associated or healthcare-associated (see Figure). (22) In total 2174/5405 (40.2%) of hospitalized patients with CDI had onset of their symptoms in the community. This proportion increased over the years. There was a delay in testing for CDI in community-onset episodes compared to hospital-onset episodes (median time between start of symptoms and CDI test four days vs one day, $P < .001$). PCR RT001 was more frequently found in patients with hospital-onset episodes, while RT023 was more frequently found in community-onset episodes. Although results from this study should be interpreted with caution as the design (i.e. only including patients with hospital-onset CDI or patients with CO-CDI who necessitated hospital admission) led to collider bias, we did demonstrate that CO-CDI episodes contribute considerably to the total CDI burden in hospitals as 6/7 surgeries, 27/50 ICU admissions and 48/107 CDI-associated deaths were reported in community-onset episodes. Surveillance programs that also target non-hospitalized patients will give a better impression of the true burden of CDI inside and outside the hospital. Extending surveillance to the community may also provide valuable information about transmission patterns and shed new light on other *C. difficile* sources, including colonized patients.

Challenges in *C. difficile* colonization studies

When performing or interpreting studies on *C. difficile* colonization, several challenges are encountered, including diagnostic procedures, representativeness of study cohorts and duration of carriage.

Diagnostic procedures

When studying CDC in humans, the first hurdle to overcome is to establish a correct diagnosis of CDC.

The various assays that are available for diagnosing CDI are also applied in studies where CDC is the condition of interest. Culture has been applied in studies investigating CDC, with or without determination of the toxin-producing potential of the recovered isolate (ie, toxigenic culture). As bacterial counts may be lower than in CDI, sensitive culture media should be used or a broth enrichment culture should be applied. (23) A major advantage of using culture is that recovered isolates can be used for (ribo)typing and transmission investigations. Nowadays most studies that do not focus on transmission patterns use NAAT as the assay of choice to detect CDC. NAAT has a good sensitivity and specificity compared to the gold standard toxigenic culture and is therefore a reasonable faster and less labor-intensive method. Most NAATs detect conserved regions within *tcdB*, and hence only toxigenic strains are detected. As both TC and NAAT detect the presence of a toxigenic *C. difficile* strain, the presence of diarrhea should be ruled out to be confident that these patients are not actual CDI patients.

Colonized patients are assumed to have toxin negative stools. Therefore, Tox A/B EIA and CCNA are an illogical choice if CDD is the condition of interest as these assays detect free toxins instead of the presence of the organisms. However, it is not always as clear-cut as that. In infants, a positive CCNA in the absence of clinical symptoms may be indicative of CDC as toxin presence does not always seem to correlate with clinical symptoms in this age group. (24) But also in adult patients with asymptomatic CDC, positive CCNA results can be found: in one study 30/77 rectal swabs from CDC patients tested positive by CCNA. (25) Despite these observations, there is still no rationale for the use of a Tox A/B EIA or CCNA in studies investigating CDD as has been previously done (26): although some colonized patients with a positive assay but absence of diarrhea can be diagnosed in this way, the majority of colonized patients will have no toxin production and will go unnoticed.

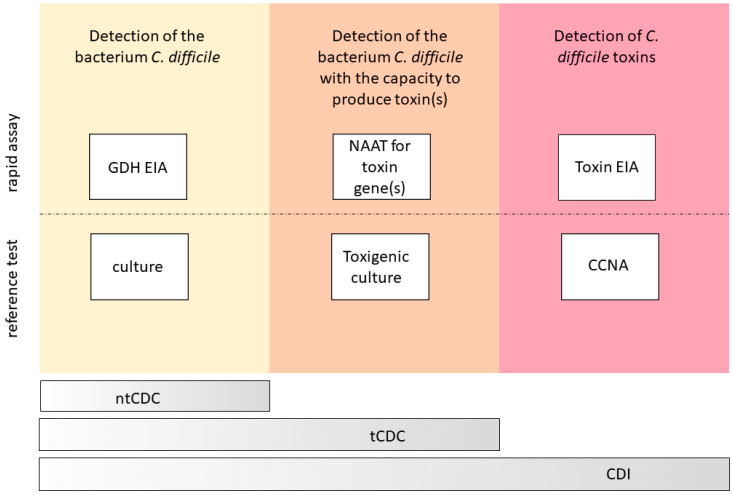


Figure 2. Assays used for detection of *C. difficile* colonization and diagnosing *C. difficile* infection. The lower bars indicate which category of assays will give positive results in these three conditions. CDI, *Clostridoides difficile* infection; CCNA, cell cytotoxicity neutralization assay; EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; NAAT, nucleic acid amplification test; ntCDC, *C. difficile* colonization with a non-toxigenic strain, TC, toxigenic culture; tCDC, *C. difficile* colonization with a toxigenic strain.

In many studies investigating CDC rectal or even perirectal swabs are used instead of stool samples. The use of (peri)rectal swabs has the advantage of obtaining samples in a timely manner without the need to wait for bowel movements. As testing for CDC in studies is often performed as part of a screening program timely results are needed, especially if isolation precautions are imposed on colonized patients. (27) Several studies have demonstrated acceptable sensitivity when culture (28, 29), or NAAT (30-32) are performed on (peri)rectal swabs. Swabs in these studies were however taken from symptomatic patients or even swabbed from *C. difficile* positive stool samples. As the mean density of *C. difficile* in stool sof asymptomatic carriers has been reported to be 100-fold lower than for CDI patients, perirectal swabs might be less suitable for detecting CDC. (33) In a screening study in asymptomatic patients, NAAT on perirectal swabs was shown to have a positive predictive value of 75.2% compared to toxigenic culture on the same perirectal swabs, with part of the NAAT positive toxigenic culture negative results explained by the receipt of *C. difficile* inhibitory antimicrobials. (34) An alternative method to enhance *C. difficile* detection from perirectal swabs in low density samples is an enrichment broth prior to NAAT testing. (35) Compared to toxigenic culture from stool samples, sensitivity and specificity for this test method from perirectal swabs were 100% and 99.1% respectively. (35) At the moment, large

studies that compare direct NAAT or TC on perirectal swabs versus on stool samples in asymptomatic patients are lacking. In many studies, the advantages of rapid and more complete sampling by the use of (peri)rectal swabs will outweigh the drawback of their possible suboptimal sensitivity. Choices for testing methods will therefore merely depend on study aims and resources, but when appraising the literature it is important to be aware of these limitations. Of note, the procedure for taking rectal swabs is often not specified in studies which makes it even more difficult to interpret if true rectal swabs (e.g., placing the swab into the rectum, rotating and removing the swab) or (non-invasive) perirectal swabs were taken.

In studies comparing infected with colonized patients, reliance on NAAT testing could unintentionally deplete the colonized group of less healthy subjects. In these studies, the absence of diarrhea is often a prerequisite for being classified as colonized instead of infected. However, CDC patients may very well develop diarrhea due to non-CDI reasons and excluding these patients from the colonized group may affect microbiota and epidemiological studies.

When estimating the risk for colonized patients to progress to CDI, a correct diagnosis of CDI is also essential. Although diagnostic guidelines have been published **(CHAPTER 3)** (36), recommended CDI testing algorithms are frequently not in place, neither in daily practice nor in studies. Data from ECDC CDI surveillance show that in 2016, ESCMID recommended algorithms were not used in 28.5% of surveillance periods in participating hospitals (ECDC 2018). In the Netherlands, most recent data show that 41% of hospitals participating in national CDI surveillance do not use an ESCMID recommended algorithm; 36% of hospitals relied on stand-alone NAAT. (37) Also in studies, reliance on stand-alone NAAT testing is common, although large studies have shown that NAAT positivity (ie without toxin positivity) does not correlate with clinical outcome and is therefore not indicative of true CDI. (2, 38) Although low cycle threshold (CT) values by NAAT testing could indicate toxin positive patients, toxin testing is still needed due to the suboptimal specificity of this approach **(CHAPTER 4).** (39) Refraining from free toxin testing can introduce misclassification of colonized patients as CDI and can inflate incidence rates. This has been shown in the LUCID study in which CDI testing policies across 60 hospitals in three European countries (UK, France, Italy) were investigated (40). In hospitals that used methods that do not detect free toxin, mean CDI positivity rates were 2.5-fold higher than in hospitals using a recommended algorithm for CDI testing. Annual CDI incidence rates were also significantly higher: 5.2/10,000 patient-days in hospitals not detecting toxin

versus 2.0/10,000 patient-days in hospitals using a recommended algorithm. (40) When CDC patients are monitored for development of subsequent CDI, omitting free toxin testing in case of suspected CDI may very well overestimate the risk to develop CDI. Still, several studies using NAAT or toxigenic culture for detection of CDC permit a positive result with the same assay to be diagnostic for CDI once the patient develops diarrhea. (41-43) For example, in the study by Blixt and colleagues in which 3605 patients were screened for CDC on admission, a high risk of developing CDI during hospitalization was found for patients colonized by toxigenic *C. difficile* (9.4% versus 2.3% in non-colonized patients). (43) However, both *C. difficile* colonization and infection were based on a positive NAAT, with the only difference the absence or presence of diarrhea at moment of testing. Studies that omit free toxin testing for CDI diagnosis mostly require that other reasonable explanations for diarrhea are absent before considering diarrhea due to *C. difficile*, but clinical judgment can be burdensome especially in situations where there is a high chance of developing diarrhea. In a study performed in an ICU setting, CDC at ICU admission was associated with a strongly increased risk for development of CDI (relative risk 10.3 compared to non-colonized patients), but as both CDC and CDI were based on NAAT testing, there is a chance that these figures are inflated due to the many other reasons for diarrhea in the ICU setting, which were probably not apparent. (44)

Given the above, it is clear that the wide range of available assays and testing strategies induce heterogeneity between studies. Interpreting study results therefore should always include a thorough assessment of methods used to diagnose CDC (and CDI). The above also highlights that not only CDI diagnosis should be based on laboratory assays in combination with clinical symptoms, but that the same holds for CDC diagnosis. Studies that are not only based on laboratory data but also include clinical evaluation have added value.

Representativeness of study cohorts

Collecting fecal samples for studies on CDC can be burdensome. In our CDD study (CHAPTER 6), over 5000 patients agreed to participate in the study, but we only received stool samples in 42% of them. Similar figures are reported in other studies that tried to obtain stool samples at hospital admission: a UK and US study managed to obtain samples in 132/227 (58%) and 320/729 (43%) of consenting patients, respectively. (15, 16) These suboptimal collection rates may impact the representativeness of study cohorts. In the US study by Leekha, patients who submitted a stool sample were older and had more often a history of recent hospitalization, antibiotic use or residency in a long-term care

facility. (16) On the other hand, in the study by Eyre, age was not different among patients who did or did not submit a stool sample, but patients who did submit stool samples more often had loose stools, a non-hematological malignancy or use of gastric acid suppressant medication. (15) To be less dependent on participants' bowel habits, the use of rectal swabs has been applied in several studies. In a large Canadian study investigating colonization on admission, rectal swabs were used in case no stool sample could be obtained. (17) With this approach, they were able to collect a sample from 5232/5422 (96%) of consenting subjects. Although this rate is significantly higher than in studies collecting stool samples, only 5422/9502 (57%) of eligible patients agreed to participate in the study. If this was due to the invasive character of the rectal swab procedure or venipuncture that was scheduled on the day of admission, remains speculative.

Moreover, a greater or smaller subset of patients of interest is classified ineligible depending on the exclusion criteria of the specific study. For example, patients admitted for palliative care or with hemodynamic instability are generally excluded. In studies using rectal swabs, additional exclusion criteria like neutropenia or thrombocytopenia are often applied. In most studies, patients with an anticipated short hospital stay are excluded, although definitions of short stay may range from 24 hours to 5 days. (27, 45) Given the subset of patients deemed ineligible and the difficulties in obtaining consent for rectal swabs or obtaining stool samples, one should consider that in most studies only a small proportion of patients on hospital admission are included in the final study cohort. In the aforementioned Canadian and US study, 5232/12304 (43%) and 320/1464 (22%) of newly admitted patients were finally included, respectively. (16, 17) Colonization rates may be influenced by this selection, although the magnitude remains unclear. Except from a possible effect on colonization rates, suboptimal inclusion rates may hamper the interpretation of studies that are investigating transmission patterns. In studies that investigate onwards transmission from colonized to symptomatic patients, new *C. difficile* introductions into the ward will be missed if not all newly admitted patients can be screened on admission. Hence, the contribution of colonized patients to *C. difficile* epidemiology may be underestimated.

The difficulties in obtaining consent and samples can be overcome if *C. difficile* screening can be implemented in another obligatory screening program. This was done in one study where patients undergoing VRE surveillance testing were also screened for *C. difficile*. (13) The drawback of this approach, however, is that screening was only performed in patients with known risk factors for VRE -which may not be the same for *C. difficile*- i.e., if admitted from another healthcare facility or if admitted to the ICU. In 2013, an intervention consisting

of detecting and isolating of CDC patients was endorsed in the Quebec Heart and Lung Institute in Quebec in response to high healthcare-associated CDI incidence rates. (27) Patients admitted directly to the ward or with an anticipated stay <24hrs were ineligible due to logistical reasons. Aside from these patients, 7599/8218 (93%) of eligible patients could be screened during the intervention. Although a comparable approach seems the most promising to achieve high inclusion rates, implementing such a program for study purposes cannot be justified in most situations.

Apart from the low inclusion rates encountered in most studies, another issue that may impact the representativeness of the results is the in- or exclusion of patients with previous CDI. In- or exclusion criteria for patients with previous CDI differ between studies and this may effect representativeness and induce heterogeneity between studies. These differing criteria may very well impact colonization rates and the risk for patients to progress to CDI among studies, as patients with previous CDI do shed spores for prolonged times and are at risk for developing recurrent CDI. (46, 47)

In the CDD study **(**CHAPTER 6**),** we also encountered the difficulties in obtaining samples. For example, we decided not to exclude patients with an anticipated short hospital stay as our goal was to estimate the total number of colonized patients at admission and to include all isolates that could be transmitted to other patients. However, our attempt to enroll all these patients was not successful as many patients were already discharged before a stool sample could be obtained. We therefore think that our work cannot be used to make definitive conclusions on the precise contribution of colonized patients in *C. difficile* epidemiology in our setting. We do think however, that we demonstrated that *C. difficile* screening in an endemic situation like ours does not detect a significant amount of introductions of *C. difficile* in the ward that are the source for new *C. difficile* infections.

Duration of carriage and study design

CDC is not a static condition, instead studies have shown that colonization is often lost without targeted interventions. (45, 48) Also, a surveillance study in healthy subjects in the community showed that among those subjects who remained *C. difficile* positive, this was often not due to retainment of the same strain. (49)

In fact, the detection of *C. difficile* in stools may not even imply true colonization, but may also be indicative of pass-through of the bacterium without it establishing true colonization.

In an experimental study, administration of a suspension of non-toxigenic *C. difficile* spores after oral vancomycin pretreatment led to persistent CDC in 44% of healthy volunteers 14-21 days after the last dose of spores. (50) However, *C. difficile* was also temporarily found in stools of patients who were not pretreated with oral vancomycin 2 days after their last dose but not beyond, indicating transient pass-through of ingested *C. difficile* rather than colonization in this group. Or, alternatively, these patients without antibiotic pretreatment did develop less intense colonization below the threshold of detection. As most studies accept a single *C. difficile* positive sample diagnostic of CDC, the group of CDC patients in most studies constitutes a heterogenic population of patients with colonization, transient carriage and pass-through. This has implications when risk factors for CDC are investigated, as risk factors for truly colonized patients may very well differ from risk factors for patients with pass-through – who might not have risk factors at all.

Also, when appraising the risk of colonized patients to progress to CDI, the case-mix of patients in studies may impact the perceived risk as patients with transient CDC might have another risk for progression than patients with longstanding CDC.

For transmission studies, this heterogeneity of the study population is probably less important. When studying introductions of *C. difficile* strains into the hospital environment and their onward transmission, the total burden of patients carrying *C. difficile* at that moment should be included, as all these patients contribute to the shedding of spores. However, infection pressure will depend on the duration of colonization and subsequent shedding of spores, too.

C. difficile colonization revisited

Data from this thesis will be combined with recent literature to elaborate on the role of *C. difficile* colonization in the epidemiology of *C. difficile*.

Role of colonized patients in *C. difficile* epidemiology: progression to CDI

Previous studies have shown that patients colonized by toxigenic strains on hospital admission have a higher risk to progress to CDI during admission, with a pooled 5.9-times higher risk than non-colonized patients. (18) In a recent large study on this topic 19112 patients were screened for CDC on admission (as part of a *C. difficile* admission screening

and isolation program) and development of CDI during or after discharge was retrieved for all colonized patients. (51) In total 7.6% of 513 colonized patients developed CDI during hospital stay (median onset of symptoms 4 days after admission), and an additional 3.6% of patients who did not develop CDI during admission eventually developed CDI after admission. Another large study in which 3605 patients were screened on admission also found that patients with tCDC were at a higher risk of developing CDI: 20/213 (9.4%) patients with tCDC versus 76/3251 (2.3%) of non-colonized patients developed CDI. (43)

In our study, we could not confirm this high risk for tCDC patiens to develop CDI. This could be due to the fact that we were only able to include a limited number of colonized patients. However, differences in study setting may also impact the risk of colonized patients to progress to CDI and should be considered here.

In contrast to the two large studies mentioned before, our study was performed in a setting with a low CDI incidence overall, and more specifically a low PCR RT027 incidence: during the study, this ribotype was not found among infected or colonized patients. In the context of high PCR RT027 infection rates, colonization rates by this ribotype appear to be increased, too. (33, 52) Patients who acquire *C. difficile* PCR RT027 during admission will more often develop symptomatic CDI than remain asymptomatically colonized. (52), indicating that the virulence of the acquired strain can affect the likelihood to develop colonization or infection. If this also implies that once colonized with PCR RT027 there is still a higher chance to progress to CDI is unclear. However, as RT027 is often acquired during hospital admission (52, 53), patients colonized by PCR RT027 may represent a subgroup of patients with recent acquisition of this strain during a previous admission. Recent acquisition of *C. difficile* in itself was shown to be more strongly associated with development of CDI than pre-existing colonization. (54)

In our study, patients were only enrolled from regular medical and surgical wards; intensive care units or bone marrow transplant units were not included. On these latter wards, several risk factors that were found to increase the risk to progress to CDI may be more prevalent, including increasing length of stay, exposure to multiple classes of antibiotics, use of opioids and cirrhosis. (51) Overrepresentation of these risk factors may explain the high risk of progression to CDI in these studies. However, the fact that a substantial part of patients admitted to these wards will eventually develop diarrhea during the course of admission (often due to a variety of causes other than CDI) may also interfere with assessing

progression to CDI and may have inflated numbers, especially if less specific methods to diagnose CDI are used.

From the above we can speculate that colonization with toxigenic *C. difficile* poses patients at a higher risk to progress to CDI if certain factors are present. These factors might either be pathogen factors like the virulence of the acquired strain (i.e. PCR RT027) or host factors like microbial perturbation through use of antibiotics.

Role of *C. difficile* colonized patients: onwards transmission

Skin and environmental contamination with *C. difficile* spores is frequently found in asymptomatic carriers. (33) Therefore, onwards transmission from these asymptomatic patients might occur, but the importance of this transmission route is also very likely to depend on the setting. In the CDD study – although hampered by methodological constraints as explained before- colonized patients did not seem to play an important role in onwards transmission. This finding contrasts with studies where 6% to 29% of new CDI cases could be linked to asymptomatic carriers (13, 55), or where the amount of exposure to colonized patients correlated with the risk to develop CDI. (43)

From the literature, it is known that different *C. difficile* ribotypes have different modes of transmission. Within hospitals, PCR RT027 strains of CDI patients are often clonal or genetically related indicating higher levels of in-hospital transmissibility. (56) Onwards transmission from colonized to infected patients was also found to be frequently due to PCR RT027. (55, 57) This may be explained by a higher transmissibility of this strain due to more profuse shedding and more effective persistence of spores in the hospital environment. (58) Alternatively, the observed increased onwards transmission for this strain may be explained by the fact that patients who acquire this strain more often develop symptoms, thereby increasing detection.

In situations where infection pressure due to PCR RT027 diminishes, one might imagine that other transmission patterns become more important, like transmission from patients who are colonized on admission with non-healthcare-associated strains. In our endemic setting, patients on hospital admission were colonized with a variety of *C. difficile* strains, indicating diverse reservoirs. However, onwards transmission from these colonized patients was not detected. Possibly, these strains may less often be transmitted or may not (directly) lead to symptoms once transmitted. When diarrhea develops after discharge, CDI may not be

captured as patients might refrain from consulting their general practitioner or may not be tested for CDI in the community setting. (59) Alternatively, our study observation may be due to incomplete sampling of admitted patients in combination with the low transmission rate for a single asymptomatic carrier. (15, 60)

When putting the above in perspective, we acknowledge that colonized patients can be a source for onwards *C. difficile* transmission especially when ribotypes with higher transmissibility are prevalent among colonized patients. In the setting with high PCR RT027 rates, studies have however shown that symptomatic patients still contribute more to transmission than colonized patients. (55) If this is also true for settings with lower PCR RT027 rates needs to be elucidated. A study performed in a setting with a low PCR RT027 rate demonstrated that new CDI cases were three times more likely to be acquired from a previous CDI case than from a colonized but fecal toxin-negative patient with diarrhea. (61) Due to the design of the study, asymptomatically colonized patients (i.e. without diarrhea) were not included. Interestingly, the overall proportion of new cases that could be explained by transmission from CDI patients or colonized diarrheal patients was low in this setting: only 4% of new CDI cases were genetically related and shared the same ward simultaneously or within 28 days. Although it is not known to what extent transmission from colonized patients could explain new CDI cases, suggesting that asymptomatic carriers accounted for the remaining 96% of new CDI cases would be overstated. Hence, based on this study and from our own experience, we assume that in endemic settings sources other than direct contact with CDI patients or asymptomatic carriers may also play an important role. One of the most important sources could be the hospital environment. For PCR RT027, it was shown that transmission is even more often transmitted via ward contamination (66%) than via direct donor-recipient contact (34%). (58) Colonized patients can contaminate their hospital environment without directly transmitting *C. difficile* to another patient and spores can persist for prolonged times. Direct onward transmission will not be detected, but this is still an important route in which colonized patients can contribute to *C. difficile* transmission.

Screening for CDC and isolation of *C. difficile* colonized patients

At the moment, infection control measures focus on symptomatic cases only. Guidelines on infection prevention in *C. difficile* do not include colonized patients (62), or mention that there are insufficient data to (a) recommend screening for asymptomatic colonization and (b) placing colonized asymptomatic patients on contact precautions. (63) Patients in the community can acquire *C. difficile* from a diverse reservoirs, including (but not limited

to) food, humans, animals and soil. When these colonized patients are admitted to the hospital, they introduce these diverse *C. difficile* strains into the hospital environment. Spores of *C. difficile* can persist in the hospital environment for long times and are difficult to kill. (64) Therefore, there seems to be a rationale for screening and isolating colonized patients at admission to prevent further spread of their persistent *C. difficile* spores into the hospital environment.

The most well-known example of a *C. difficile* screening program comes from a Canadian tertiary hospital. In this hospital, screening for CDC and isolation of colonized patients has been performed in response to high endemic CDI incidence rates. (27) All patients admitted through the emergency department were screened for CDC on admission using rectal swabs. Patients identified as colonized were placed under infection control measures resembling those for CDI. A significant decline in healthcare-associated CDI and infections due to PCR RT027 was noted after implementation of this strategy.

With the identification of risk factors for CDC on admission, screening may also be targeted to high risk groups only, making this approach less labor- and cost intensive. Previous hospitalization, gastric acid suppression, tube feeding, and corticosteroid use were identified as independent predictors of CDC in a recent meta-analysis (65), but risk factors for community-onset colonization (i.e. being colonized at hospital admission) were not separated from risk factors for hospital acquired colonization, though only the former are of interest for an admission screening program. Also, a distinction between colonization by toxigenic versus non-toxigenic strains was not made. Only a few studies have specifically investigated risk factors for tCDC on admission and reported recent hospitalization, chronic dialysis, corticosteroid use (16), older age, higher frailty scores (66), higher number of comorbidities, female sex and residential proximity to livestock farms as independent risk factors. (67) Risk factors for tCDC on admission will however largely depend on the community surrounding the hospital and case-mix of patients that are admitted. Knowledge of these specific risk factors may enable institutions to tailor screening to risk groups in their hospitals. This approach was applied in four US hospitals where admission screening for *C. difficile* was performed as an infection control initiative. (68) Only patients who had been previously hospitalized within two months, and/or had a history of CDI, and/or were in a long-term care facility in the prior six months were screened for tCDC on admission using perirectal swabs. Based on a pilot study in one of the participating hospitals the authors anticipated that they would detect 78% of all colonized patients while only testing 30% of admissions. Colonized patients were placed under infection control measures. A

statistically significant decline in hospital-onset CDI rates in these hospital was noted after implementation of this screening program. (68)

However, a major shortcoming of studies investigating *C. difficile* screening programs is that these do not show how the decrease in CDI incidence rates was achieved. (69) In these studies, isolates from CDI patients and colonized patients have not been investigated by whole genome sequencing or another discriminative typing method to determine their relatedness. Also, the numbers of colonized patients progressing from colonization to CDI have not been reported. Therefore, it is unclear if decreasing CDI incidence rates were due to (1) less progression from colonization to symptomatic CDI, (2) less onwards transmission from colonized patients, or (3) less transmission from known CDI cases. (Figure 3) As colonization status was disclosed to treating physicians, they might have restricted antibiotic use in colonized patients to decrease the chance of progression to CDI. Also, compliance with infection control measures may have increased as a result of an increased awareness associated with the introduction of the screening program, thereby diminishing spread from CDI patients. In the study by Longtin, an increased hand hygiene compliance was reported during the intervention period (suggesting that this but probably also other unmeasured confounders may have contributed to the decline in CDI rates). (27) It is crucial to understand which mechanisms led to the decrease in CDI incidence rates and decrease in NAP1/027 cases, especially to determine if CDC patients really need to be isolated, or whether increased awareness or only identifying CDC patients (but not isolating them) may suffice. The usefulness of a screening and isolation program will also largely depend on the epidemic setting. As discussed before, we did not detect colonized patients to be an important source for onwards *C. difficile* transmission in an endemic setting. **(CHAPTER 6)** Screening and isolation of colonized patients in an endemic setting may however still (indirectly) affect CDI incidence rates if less contamination of the hospital environment leads to less *C. difficile* acquisition and development of CDI in vulnerable patients. However, the question is if interventions intended to reduce transmission within hospitals are the most important in reducing CDI incidence rates. Two large multicentre studies have shown disappointing results from enhanced environmental disinfection on CDI incidence rates (70-72), despite a decreased recovery of *C. difficile* from high-touch surfaces in CDI rooms. (72) Even more, the believe in contact precautions for CDI patients as an important tool in CDI prevention may not always be justified. In a Swiss hospital with endemic CDI rates and a low proportion of hypervirulent ribotypes, contact precautions for CDI patients who were not severely incontinent were discontinued, except for the use of dedicated toilets. (73) Only two proven *C. difficile* transmission events were identified over a decade of experience in this hospital.

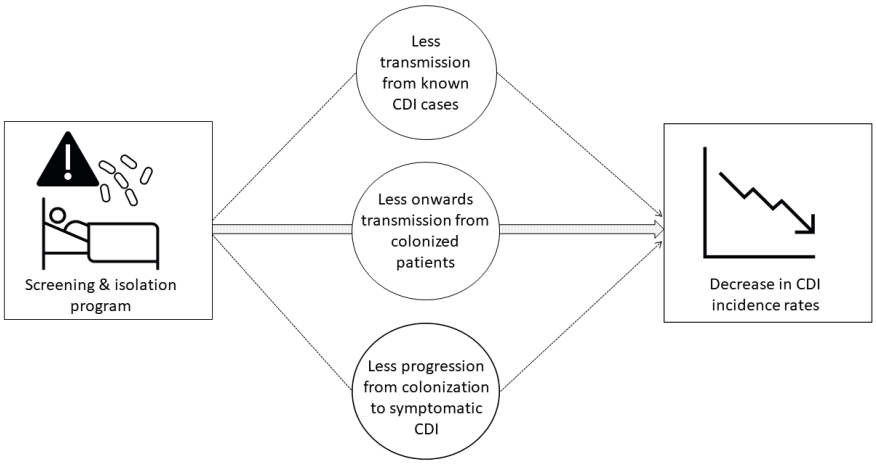


Figure 3. Mechanisms that may lead to a decrease in CDI incidence rates after implementation of screening for *C. difficile* colonization and isolation of colonized patients.
CDI, *Clostridoides difficile* infection

Detecting colonized patients may still aid in lowering CDI incidence rates, if targeted interventions for this group other than isolation precautions may decrease CDI incidence rates. This intervention may consist of antimicrobial stewardship programs (ASPs) focused specifically on colonized patients. ASPs that reduce inappropriate antimicrobials use are effective in reducing rates of CDI, especially in geriatric settings. (74) However, ASPs targeted at CDC patients only have not been studied. A subset of colonized patients is comprised of patients with previous CDI who remained colonized afterwards. These patients are known to be at a higher risk for (recurrent) CDI when treated with antibiotics. (75) ASPs focused specifically on these patients may thus be effective. However, another subset of colonized patients comprises patients who are long-term colonized, and who may not have a high risk to progress to CDI. Therefore, ASPs targeted on colonized patients as a whole group may not be effective. Of note, recent acquisition of *C. difficile* has been shown to be a risk factor for CDI. (54) Shifting the focus of ASPs to colonized patients only may therefore lead to unsatisfactory results, as non-colonized patients who acquire *C. difficile* during admission are not targeted by these programs, while indeed these patients may be at an increased risk to develop CDI. Other strategies that are currently investigated as prophylactic interventions for prevention of *C. difficile* infection in at-risk patients might also be suitable to prevent CDI progression in colonized patients, for example a toxin-based *C. difficile* vaccine (phase III CLOVER trial, NCT03090191, study completed December

2021, results awaited). If microbiota-based interventions like FMT capsules during antibiotic treatment or agents that bind and inactivate concomitantly administered antibiotics (such as ribaxamase and DAV-132), are also helpful in already colonized patients has to be awaited.

Apart from the fact that the relevance and mode of action of screening programs needs to be established better among different situations, there are also some unintended consequences of a screening and isolation program that need to be taken into account.

Implementing a screening and isolation program will lead to more patients cared for under contact precautions, with adverse outcomes like less patient- healthcare worker contact and a decreased patient satisfaction with care. (76) Also, the knowledge of CDC status may urge physicians to treat colonized patients with antibiotics once they develop diarrhea, despite the fact that diarrhea can be due to a variety of other causes. Treatment of colonized patients may however be more harmful than beneficial. Already in 1992, it was discovered that vancomycin treatment of colonized patients was temporarily effective, but also associated with a significantly higher rate of *C. difficile* carriage two months after treatment compared to placebo treated carriers. (48) In a recent small trial, NAAT-positive, toxin enzyme immunoassay (EIA)-negative patients were randomized to oral vancomycin or placebo. Oral vancomycin did not result in long-term clearance of *C. difficile*, but did disturb the microbiota, and was associated with colonization/shedding of vancomycin-resistant enterococci. (77)

In conclusion, reports have demonstrated decreases in CDI rates after implementation of screening and isolation of CDC patients. However, these reports did not show how this decrease was achieved. Also, the generalizability to endemic settings has not been shown. Although detection of colonized patients may also enable ASPs focused specifically on colonized patients, this approach has not been studied yet, and may in fact be less effective than universal ASPs. Given these limitations and the possible adverse consequences of a screening and isolation program, screening for asymptomatic CDC at hospital admission should not be implemented in routine care.

Instead, we should accept that *C. difficile* is abundant both in the hospital environment and the community setting, and can be acquired easily. In my opinion, it is better to focus on decreasing CDI susceptibility (e.g. by general ASPs) while complying with general infection prevention measures to prevent further spread from *C. difficile*, especially in settings with many susceptible patients like healthcare facilities.

Future Perspectives

In this paragraph, I will use the data from this thesis and the literature to elaborate on:

- Future CDI surveillance
- Disturbed microbiota: associated diseases and drug efficacy
- Microbiota modulating interventions

Future CDI surveillance

In this thesis, we have mainly discussed the role of colonized patients in epidemiology of CDI in the hospital. We have concluded that in an endemic setting, focusing on colonized patients will not have a major impact on CDI incidence rates. Should we then focus on other sources for *C. difficile* acquisition? Many *C. difficile* reservoirs exist, including domestic animals, farm animals, wild animals, food, water and soil. Previously undetected ribotypes can still emerge from these reservoirs, including more virulent strains or strains with higher transmissibility. Once a patient introduces such a *C. difficile* strain into the hospital, it may be detected through the observation of higher CDI incidence rates or suspected transmission events. The PCR RT826 cluster that we detected in our multicenter study is a good example of this and confirms the importance of sentinel CDI surveillance (supplemented with molecular typing in case of suspected transmission events). However, as sentinel surveillance is restricted to the hospital and CDI awareness in the community is still limited (59), CDI cases and even clusters of CDI cases in the community may be overlooked. This is problematic when studying transmission, as exposure to most *C. difficile* reservoirs (like animals, soil and food) occurs in the community. If transmission events close to the source are not detected, tracing back the source and transmission route once that specific strain is introduced into the hospital is almost impossible. Extending *C. difficile* surveillance to the community setting might enable detection of transmission within the community and possibly point to specific sources. However, the usefulness of a surveillance program in the community will depend on the capture rate of all CDI events. As most patients do not consult their general physician for short-term diarrhea and testing for pathogens in patients with diarrhea for less than 10 days or who are not severely ill is discouraged in The Netherlands (NHG standaard acute diarree 2014), we suspect that only a minority of CDI cases will be detected. Hence, suboptimal tracking of *C. difficile* transmission would occur thereby limiting the yield of a community surveillance program. Thus, we should settle for the second-best option of continuing surveillance in healthcare settings to detect increases in CDI incidence rates, clusters or outbreaks. In unforeseen

events, like community-acquired CDI with 'hypervirulent' or new ribotypes, additional investigations should be performed to evaluate potential transmission in the community or detect potential community sources.

Disturbed microbiota: associated diseases and drug efficacy

In this thesis we have previously focused on the risk for colonized patients to develop CDI, but what other consequences does colonization have for the involved individual? In this paragraph we will focus on conditions that are associated with a disturbed microbiota and on drugs whose efficacy may depend on the gut microbiota composition.

CDI is the textbook example of a disease that is caused by a disturbed microbiota. The overall microbiota composition of colonized patients differs from that of patients with symptomatic CDI, but it is also characterized by a decreased species richness and decreased microbial diversity compared to non-colonized patients or healthy controls. (9) (CHAPTER 5) I therefore believe that the presence of CDC can be regarded as an indicator of a certain degree of a disturbed microbiota. In addition, colonization in itself may possibly further disturb the microbiota, as was demonstrated using an *in vitro* model. (78) In this model, co-cultivation of *C. difficile* strains with fecal microbiota led to a decrease in richness and diversity, with a more pronounced effect in already disturbed microbiota. (78) 'Gut dysbiosis' has gained a lot of attention during the last decade, and was reported to be associated with intestinal disorders like inflammatory bowel disease and irritable bowel syndrome and the development of colorectal cancer. (79-82) Moreover, 'gut dysbiosis' is thought to exert effects beyond the gut, for example by influencing the risk factors for metabolic syndrome. (83) In addition, neurological diseases like Parkinson's disease and multiple sclerosis and psychiatric disorders are thought to be linked to 'gut dysbiosis' via the so-called hypothetical gut-brain axis. (84, 85) Although the causal direction of association between a disturbed microbiota and disease cannot be derived from observational studies in humans, animal models suggest that the microbiota is truly involved in pathogenesis of most of above mentioned conditions. (86) Assuming that a disturbed microbiota actually has a role in pathophysiology, one might wonder if patients with CDC are more prone to develop other 'dysbiosis' associated conditions, as they already have a disturbed microbiota. However, the exact microbiota changes will determine which conditions might be associated. A very typical example of this is the possible association between colibactin-producing *E. coli* and colorectal carcinogenesis. Colibactin-producing *E. coli*, also known as polyketide synthase-positive (pks+) *E. coli* are suspected to contribute to

colorectal carcinogenesis by the production of the genotoxin colibactin which induces double-strand DNA breaks. These pks+ *E. coli* are abundant in patients with recurrent CDI (87), but it is still unknown if this also holds for CDC patients.

Besides playing a possible role in the pathophysiology of certain diseases, the microbiota can also play a role in the effectiveness of certain drugs, for example by providing the required metabolism, such as for lactulose. (88) Another important illustration of altered drug efficacy is the potential influence of gut microbiota composition on anticancer activity of checkpoint inhibitors. Checkpoint inhibitors are immunomodulators that have dramatically changed the therapeutic landscape in several cancer types during the last decade. Checkpoint inhibitors work by blocking inhibitory checkpoints (e.g. programmed cell death-1 or programmed cell death ligand-1) thereby promoting immune-mediated elimination of tumor cells. Studies have shown that recent antibiotic use before starting checkpoint inhibitor therapy is associated with decreased progression free survival and overall survival (89-92), indicating that antibiotic induced disturbances modulate the immune response and dampen the effect of checkpoint inhibitors. Evidence for a causal relationship comes from studies in tumor bearing germ free mice: fecal microbiome transplantation (FMT) demonstrated that mice transplanted with stool from patients responding to checkpoint inhibitors had significantly reduced tumor growth compared to those transplanted with stool from patients who were non-responders. (93, 94) Recently, studies have been undertaken to evaluate which gut microbiota members are associated with clinical response in patients treated with checkpoint inhibitors. Although variable results were obtained, some bacterial species including *Akkermansia muciniphilia* and *Ruminococcaceae* were repeatedly found to be associated with favorable outcomes. (95) Given the disturbed gut microbiota composition and lower bacterial diversity in CDC patients, it would be interesting to assess if these patients represent a group that is at risk for decreased effectiveness of checkpoint inhibitors.

Microbiota modulating interventions

The evident association of CDI with a perturbed gut microbiota prompts the question if actions to restore the gut microbiota could be beneficial prior to the development of overt disease. One method to restore the perturbed microbiota in CDC subjects could be fecal microbiota transplantation (FMT), i.e. the transfer of faecal material from a healthy donor to the colonized subject. At the moment, FMT is well-known for its high efficacy in the treatment of recurrent CDI (rCDI). (96, 97) Administration of stools of healthy donors

results in a dramatic increase in microbiota diversity and resolution of symptoms in the majority of these patients, with reported success rates of more than 90%. (98). A drawback of FMT is the potential transfer of multidrug resistant bacteria, procarcinogenic bacteria and unrecognized pathogens. Therefore, there is a need for live biotherapeutic products, which are standardized and reproducible agents composed of defined consortia of isolated microbial strains. However, development of an effective live biotherapeutic product to replace FMT is not straightforward as not all mechanisms underlying the efficacy of FMT are well known. Although reconstitution of a robust and diverse gut microbiota is thought to explain at least part of the success of FMT, other components of the donor stool infusion like bacteriophages, metabolites and small molecules may also contribute to its effect. (99) Of note, the administration of simply a variety of bacterial strains without complete understanding of their function may have unanticipated adverse effects, as was reported in some studies on probiotics. (100, 101) At the moment, two live biotherapeutic products, RBX2660 and SER109, have been investigated in phase III trials for use in patients with recurrent CDI who had resolution of symptoms after treatment with standard-of-care antibiotics. Subsequent administration of the live biotherapeutic product reduced the risk of recurrence compared to placebo. (102, 103) Another live biotherapeutic product containing eight *Clostridia* strains was shown to be safe and well-tolerated and able to colonize the gut of healthy volunteers in a phase 1a/b study, but studies on efficacy have to be awaited. (104) In the future, these products may become an alternative for FMT in the treatment of rCDI. Yet, it is unclear if FMT is able to protect colonized patients from progression to CDI. The existence of patients with persistent colonization suggest that these patients' microbiomes are already less permissive towards CDI. Further elucidation of the gut microbiota composition and associated metabolic characteristics in preferably persistent carriers may be used to more specifically develop probiotic or prebiotic therapeutics against (progression to) CDI. A recent study compared not only the gut microbiota but also the gut metabolome of CDI patients and colonized subjects. (105) Compared to CDI patients, colonized patients' microbiota was enriched with species in the class *Clostridia* and their metabolomes were enriched with the carbohydrates sucrose, rhamnose and lactulose, which are non-utilizable by *C. difficile*. Therefore, they concluded that carbohydrate metabolism by other commensal *Clostridia* may prevent CDI by inhibiting *C. difficile* proliferation. (105) Hypothetically, this commensal metabolism can be used as a more specific tool against CDI, for example by administrating 'microbial accessible carbohydrates' to prevent *C. difficile* proliferation and decrease the risk of CDI in colonized subjects.

The successful implementation of FMT as a therapy for rCDI has encouraged research into FMT as a potential therapy for many other 'dysbiosis' related conditions like inflammatory bowel disease, irritable bowel syndrome, hepatic encephalopathy and autism. (106) Also, several clinical trials are currently performed to study the effect of FMT in metastatic melanoma, lung cancer and renal cell cancer for patients who are not (anymore) responding to checkpoint inhibitor therapy. Depending on the results of the currently performed trials, FMT could possibly be used to treat other conditions beyond rCDI in the future. At the moment, there is no place for FMT/microbiota modulating interventions in CDC patients, as it is yet unknown if restoration of their gut microbiota protects against CDI or other conditions that are associated with a disturbed microbiota. However, the detection of CDC should prompt awareness of a probable associated disturbed microbiota and I suggest that medication that could further disturb the microbiota should cautiously be prescribed in these patients.

C. difficile colonized patients remain a group of special interest for future research. Further mechanistic studies may analyze which factors beyond the gut microbiota composition (e.g. gut metabolites, immunologic factors) allow for colonization whilst protecting from infection. Possibly, this could lead to new treatment or preventive modalities for CDI. On the other hand, epidemiologic studies may shed light on the long-term consequences of CDC and may elucidate possible relations with conditions that are associated with a disturbed gut microbiota.

References

1. Crobach MJ, Dekkers OM, Wilcox MH, Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): data review and recommendations for diagnosing Clostridium difficile-infection (CDI). Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2009;15(12):1053-66.
2. Planche TD, Davies KA, Coen PG, Finney JM, Monahan IM, Morris KA, et al. Differences in outcome according to Clostridium difficile testing method: a prospective multicentre diagnostic validation study of C difficile infection. The Lancet Infectious Diseases. 2013;13(11):936-45.
3. Krutova M, Wilcox MH, Kuijper EJ. The pitfalls of laboratory diagnostics of Clostridium difficile infection. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2018;24(7):682-3.
4. Drudy D, Calabi E, Kyne L, Sougioultzis S, Kelly E, Fairweather N, et al. Human antibody response to surface layer proteins in Clostridium difficile infection. FEMS Immunol Med Microbiol. 2004;41(3):237-42.
5. Bruxelle JF, Mizrahi A, Hoys S, Collignon A, Janoir C, Pechine S. Immunogenic properties of the surface layer precursor of Clostridium difficile and vaccination assays in animal models. Anaerobe. 2016;37:78-84.
6. Péchiné S, Janoir C, Boureau H, Gleizes A, Tsapis N, Hoys S, et al. Diminished intestinal colonization by Clostridium difficile and immune response in mice after mucosal immunization with surface proteins of Clostridium difficile. Vaccine. 2007;25(20):3946-54.
7. Kyne L, Warny M, Qamar A, Kelly CP. Asymptomatic carriage of Clostridium difficile and serum levels of IgG antibody against toxin A. N Engl J Med. 2000;342(6):390-7.
8. Crobach MJT, Vernon JJ, Loo VG, Kong LY, Pechine S, Wilcox MH, et al. Understanding Clostridium difficile Colonization. Clinical microbiology reviews. 2018;31(2).
9. Zhang L, Dong D, Jiang C, Li Z, Wang X, Peng Y. Insight into alteration of gut microbiota in Clostridium difficile infection and asymptomatic C. difficile colonization. Anaerobe. 2015;34:1-7.
10. Vincent C, Miller MA, Edens TJ, Mehrotra S, Dewar K, Manges AR. Bloom and bust: intestinal microbiota dynamics in response to hospital exposures and Clostridium difficile colonization or infection. Microbiome. 2016;4:12.
11. Eyre DW, Cule ML, Wilson DJ, Griffiths D, Vaughan A, O'Connor L, et al. Diverse sources of C. difficile infection identified on whole-genome sequencing. N Engl J Med. 2013;369(13):1195-205.
12. Walker AS, Eyre DW, Wyllie DH, Dingle KE, Harding RM, O'Connor L, et al. Characterisation of Clostridium difficile hospital ward-based transmission using extensive epidemiological data and molecular typing. PLoS Med. 2012;9(2):e1001172.

13. Curry SR, Muto CA, Schlackman JL, Pasculle AW, Shutt KA, Marsh JW, et al. Use of multilocus variable number of tandem repeats analysis genotyping to determine the role of asymptomatic carriers in Clostridium difficile transmission. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2013;57(8):1094-102.
14. Clabots CR, Johnson S, Olson MM, Peterson LR, Gerding DN. Acquisition of Clostridium difficile by hospitalized patients: evidence for colonized new admissions as a source of infection. J Infect Dis. 1992;166(3):561-7.
15. Eyre DW, Griffiths D, Vaughan A, Golubchik T, Acharya M, O'Connor L, et al. Asymptomatic Clostridium difficile colonisation and onward transmission. PLoS One. 2013;8(11):e78445.
16. Leekha S, Aronhalt KC, Sloan LM, Patel R, Orenstein R. Asymptomatic Clostridium difficile colonization in a tertiary care hospital: admission prevalence and risk factors. Am J Infect Control. 2013;41(5):390-3.
17. Kong LY, Dendukuri N, Schiller I, Bourgault AM, Brassard P, Poirier L, et al. Predictors of asymptomatic Clostridium difficile colonization on hospital admission. Am J Infect Control. 2015;43(3):248-53.
18. Zacharioudakis IM, Zervou FN, Pliakos EE, Ziakas PD, Mylonakis E. Colonization with toxinogenic C. difficile upon hospital admission, and risk of infection: a systematic review and meta-analysis. Am J Gastroenterol. 2015;110(3):381-90; quiz 91.
19. Knetsch CW, Connor TR, Mutreja A, van Dorp SM, Sanders IM, Browne HP, et al. Whole genome sequencing reveals potential spread of Clostridium difficile between humans and farm animals in the Netherlands, 2002 to 2011. Euro Surveill. 2014;19(45):20954.
20. Wilcox MH, Mooney L, Bendall R, Settle CD, Fawley WN. A case-control study of community-associated Clostridium difficile infection. J Antimicrob Chemother. 2008;62(2):388-96.
21. Bauer MP, Veenendaal D, Verhoef L, Bloembergen P, van Dissel JT, Kuijper EJ. Clinical and microbiological characteristics of community-onset Clostridium difficile infection in The Netherlands. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2009;15(12):1087-92.
22. European Centre for Disease Prevention and Control. European Surveillance of Clostridium difficile infections. Surveillance protocol version 2.2. Stockholm, ECDC; 2015. 2015.
23. Connor MC, McGrath JW, McMullan G, Marks N, Fairley DJ. Development of an optimized broth enrichment culture medium for the isolation of Clostridium difficile. Anaerobe. 2018;54:92-9.
24. Bolton RP, Tait SK, Dear PR, Losowsky MS. Asymptomatic neonatal colonisation by Clostridium difficile. Arch Dis Child. 1984;59(5):466-72.
25. Schweitzer L, Gervais P, Paquet-Bolduc B, Loo VG, Longtin Y. Detection of Free Toxin B in the Stool of Asymptomatic Clostridioides difficile Carriers by the Cell Cytotoxicity Neutralization Assay. Open Forum Infect Dis. 2021;8(6):ofab209.

26. Bruminhent J, Wang ZX, Hu C, Wagner J, Sunday R, Bobik B, et al. Clostridium difficile colonization and disease in patients undergoing hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2014;20(9):1329-34.
27. Longtin Y, Paquet-Bolduc B, Gilca R, Garenc C, Fortin E, Longtin J, et al. Effect of Detecting and Isolating Clostridium difficile Carriers at Hospital Admission on the Incidence of C difficile Infections: A Quasi-Experimental Controlled Study. JAMA internal medicine. 2016;176(6):796-804.
28. Rogers DS, Kundrapu S, Sunkesula VC, Donskey CJ. Comparison of perirectal versus rectal swabs for detection of asymptomatic carriers of toxigenic Clostridium difficile. J Clin Microbiol. 2013;51(10):3421-2.
29. McFarland LV, Coyle MB, Kremer WH, Stamm WE. Rectal swab cultures for Clostridium difficile surveillance studies. J Clin Microbiol. 1987;25(11):2241-2.
30. Kundrapu S, Sunkesula VC, Jury LA, Sethi AK, Donskey CJ. Utility of perirectal swab specimens for diagnosis of Clostridium difficile infection. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;55(11):1527-30.
31. Jazmati N, Kirpal E, Piepenbrock E, Stelzer Y, Vehreschild M, Seifert H. Evaluation of the Use of Rectal Swabs for Laboratory Diagnosis of Clostridium difficile Infection. J Clin Microbiol. 2018;56(7).
32. Skyum F, Chen M, Mogensen CB. Evaluation of a new fast in-house Real-Time PCR assay for detecting both Norovirus and toxigenic Clostridium difficile using fecal sample and rectal swab. Am J Infect Control. 2021.
33. Riggs MM, Sethi AK, Zabarsky TF, Eckstein EC, Jump RL, Donskey CJ. Asymptomatic carriers are a potential source for transmission of epidemic and nonepidemic Clostridium difficile strains among long-term care facility residents. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2007;45(8):992-8.
34. Patel PA, Schora DM, Singh K, Peterson LR. Validation of Active Surveillance Testing for Clostridium difficile Colonization Using the cobas Cdiff Test. J Clin Microbiol. 2018;56(4).
35. Curry SR, Schlackman JL, Hamilton TM, Henderson TK, Brown NT, Marsh JW, et al. Perirectal swab surveillance for Clostridium difficile by use of selective broth preamplification and real-time PCR detection of tcdB. J Clin Microbiol. 2011;49(11):3788-93.
36. Kelly CR, Fischer M, Allegretti JR, LaPlante K, Stewart DB, Limketkai BN, et al. ACG Clinical Guidelines: Prevention, Diagnosis, and Treatment of Clostridioides difficile Infections. Am J Gastroenterol. 2021;116(6):1124-47.
37. Vendrik KEW. Fourteenth Annual Report of the National Reference Laboratory for Clostridioides difficile and results of the sentinel surveillane. 2021.
38. Polage CR, Gyorke CE, Kennedy MA, Leslie JL, Chin DL, Wang S, et al. Overdiagnosis of Clostridium difficile Infection in the Molecular Test Era. JAMA internal medicine. 2015;175(11):1792-801.

39. Davies KA, Planche T, Shetty N, Wren M, Crook D, Wilcox M. Toxin Gene Nucleic Acid Amplification Test Cycle Threshold Result is Associated with Severity of C. difficile Infection and Poor Patient Outcomes ID Week; San Diego, CA2015.
40. Davies K, Davis G, Barbut F, Eckert C, Petrosillo N, Wilcox MH. Variability in testing policies and impact on reported Clostridium difficile infection rates: results from the pilot Longitudinal European Clostridium difficile Infection Diagnosis surveillance study (LuCID). Eur J Clin Microbiol Infect Dis. 2016;35(12):1949-56.
41. Hung YP, Lin HJ, Wu TC, Liu HC, Lee JC, Lee CI, et al. Risk factors of fecal toxigenic or non-toxigenic Clostridium difficile colonization: impact of Toll-like receptor polymorphisms and prior antibiotic exposure. PLoS One. 2013;8(7):e69577.
42. Bliss DZ, Johnson S, Savik K, Clabots CR, Willard K, Gerding DN. Acquisition of Clostridium difficile and Clostridium difficile-associated diarrhea in hospitalized patients receiving tube feeding. Ann Intern Med. 1998;129(12):1012-9.
43. Blixt T, Gradel KO, Homann C, Seidelin JB, Schonning K, Lester A, et al. Asymptomatic carriers contribute to nosocomial Clostridium difficile infection: a cohort study of 4508 patients. Gastroenterology. 2017.
44. Tschudin-Sutter S, Carroll KC, Tamma PD, Sudekum ML, Frei R, Widmer AF, et al. Impact of Toxigenic Clostridium difficile Colonization on the Risk of Subsequent C. difficile Infection in Intensive Care Unit Patients. Infect Control Hosp Epidemiol. 2015;36(11):1324-9.
45. Samore MH, DeGirolami PC, Tlucko A, Lichtenberg DA, Melvin ZA, Karchmer AW. Clostridium difficile colonization and diarrhea at a tertiary care hospital. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1994;18(2):181-7.
46. Sethi AK, Al-Nassir WN, Nerandzic MM, Bobulsky GS, Donskey CJ. Persistence of skin contamination and environmental shedding of Clostridium difficile during and after treatment of C. difficile infection. Infect Control Hosp Epidemiol. 2010;31(1):21-7.
47. Kelly CP. Can we identify patients at high risk of recurrent Clostridium difficile infection? Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2012;18 Suppl 6:21-7.
48. Johnson S, Homann SR, Bettin KM, Quick JN, Clabots CR, Peterson LR, et al. Treatment of asymptomatic Clostridium difficile carriers (fecal excretors) with vancomycin or metronidazole. A randomized, placebo-controlled trial. Ann Intern Med. 1992;117(4):297-302.
49. Kato H, Kita H, Karasawa T, Maegawa T, Koino Y, Takakuwa H, et al. Colonisation and transmission of Clostridium difficile in healthy individuals examined by PCR ribotyping and pulsed-field gel electrophoresis. J Med Microbiol. 2001;50(8):720-7.
50. Villano SA, Seiberling M, Tatarowicz W, Monnot-Chase E, Gerding DN. Evaluation of an oral suspension of VP20621, spores of nontoxigenic Clostridium difficile strain M3, in healthy subjects. Antimicrob Agents Chemother. 2012;56(10):5224-9.

51. Poirier D, Gervais P, Fuchs M, Roussy JF, Paquet-Bolduc B, Trottier S, et al. Predictors of Clostridioides difficile Infection Among Asymptomatic, Colonized Patients: A Retrospective Cohort Study. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2020;70(10):2103-210.
52. Loo VG, Bourgault AM, Poirier L, Lamothe F, Michaud S, Turgeon N, et al. Host and pathogen factors for Clostridium difficile infection and colonization. N Engl J Med. 2011;365(18):1693-703.
53. Dubberke ER, Reske KA, Seiler S, Hink T, Kwon JH, Burnham CA. Risk Factors for Acquisition and Loss of Clostridium difficile Colonization in Hospitalized Patients. Antimicrob Agents Chemother. 2015;59(8):4533-43.
54. Shim JK, Johnson S, Samore MH, Bliss DZ, Gerding DN. Primary symptomless colonisation by Clostridium difficile and decreased risk of subsequent diarrhoea. The Lancet. 1998;351(9103):633-6.
55. Kong LY, Eyre DW, Corbeil J, Raymond F, Walker AS, Wilcox MH, et al. Clostridium difficile: Investigating Transmission Patterns Between Infected and Colonized Patients Using Whole Genome Sequencing. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2019;68(2):204-9.
56. Martin JSH, Eyre DW, Fawley WN, Griffiths D, Davies K, Mawer DPC, et al. Patient and strain characteristics associated with Clostridium difficile transmission and adverse outcomes. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018.
57. Donskey CJ, Sunkesula VCK, Stone ND, Gould CV, McDonald LC, Samore M, et al. Transmission of Clostridium difficile from asymptomatically colonized or infected long-term care facility residents. Infect Control Hosp Epidemiol. 2018;39(8):909-16.
58. Kumar N, Miyajima F, He M, Roberts P, Swale A, Ellison L, et al. Genome-Based Infection Tracking Reveals Dynamics of Clostridium difficile Transmission and Disease Recurrence. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2016;62(6):746-52.
59. Hensgens MP, Dekkers OM, Demeulemeester A, Buiting AG, Bloembergen P, van Benthem BH, et al. Diarrhoea in general practice: when should a Clostridium difficile infection be considered? Results of a nested case-control study. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2014;20(12):O1067-74.
60. Durham DP, Olsen MA, Dubberke ER, Galvani AP, Townsend JP. Quantifying Transmission of Clostridium difficile within and outside Healthcare Settings. Emerg Infect Dis. 2016;22(4):608-16.
61. Mawer DPC, Eyre DW, Griffiths D, Fawley WN, Martin JSH, Quan TP, et al. Contribution to Clostridium Difficile Transmission of Symptomatic Patients With Toxigenic Strains Who Are Fecal Toxin Negative. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017;64(9):1163-70.

62. Tschudin-Sutter S, Kuijper EJ, Durovic A, Vehreschild M, Barbut F, Eckert C, et al. Guidance document for prevention of Clostridium difficile infection in acute healthcare settings. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2018;24(10):1051-4.

63. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, et al. Clinical Practice Guidelines for Clostridium difficile Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018;66(7):987-94.

64. Reigadas E, Vázquez-Cuesta S, Villar-Gómara L, Onori R, Alcalá L, Marín M, et al. Role of Clostridioides difficile in hospital environment and healthcare workers. Anaerobe. 2020;63:102204.

65. Anjewierden S, Han Z, Brown AM, Donskey CJ, Deshpande A. Risk factors for Clostridioides difficile colonization among hospitalized adults: A meta-analysis and systematic review. Infect Control Hosp Epidemiol. 2021;42(5):565-72.

66. Meltzer E, Smollan G, Huppert A, Fluss R, Tal I, Gilboa M, et al. Universal screening for Clostridioides difficile in a tertiary hospital: risk factors for carriage and clinical disease. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2019;25(9):1127-32.

67. Muñoz-Price LS, Hanson R, Singh S, Nattinger AB, Penlesky A, Buchan BW, et al. Association Between Environmental Factors and Toxigenic Clostridioides difficile Carriage at Hospital Admission. JAMA Netw Open. 2020;3(1):e1919132.

68. Peterson LR, O'Grady S, Keegan M, Fisher A, Zelencik S, Kufner B, et al. Reduced Clostridioides difficile infection in a pragmatic stepped-wedge initiative using admission surveillance to detect colonization. PLoS One. 2020;15(3):e0230475.

69. Crobach MJ, Terveer EM, Kuijper EJ. Effect of Detecting and Isolating Asymptomatic Clostridium difficile Carriers. JAMA internal medicine. 2016;176(10):1572-3.

70. Anderson DJ, Chen LF, Weber DJ, Moehring RW, Lewis SS, Triplett PF, et al. Enhanced terminal room disinfection and acquisition and infection caused by multidrug-resistant organisms and Clostridium difficile (the Benefits of Enhanced Terminal Room Disinfection study): a cluster-randomised, multicentre, crossover study. Lancet. 2017;389(10071):805-14.

71. Anderson DJ, Moehring RW, Weber DJ, Lewis SS, Chen LF, Schwab JC, et al. Effectiveness of targeted enhanced terminal room disinfection on hospital-wide acquisition and infection with multidrug-resistant organisms and Clostridium difficile: a secondary analysis of a multicentre cluster randomised controlled trial with crossover design (BETR Disinfection). Lancet Infect Dis. 2018;18(8):845-53.

72. Ray AJ, Deshpande A, Fertelli D, Sitzlar BM, Thota P, Sankar CT, et al. A Multicenter Randomized Trial to Determine the Effect of an Environmental Disinfection Intervention on the Incidence of Healthcare-Associated Clostridium difficile Infection. Infect Control Hosp Epidemiol. 2017;38(7):777-83.

73. Widmer AF, Frei R, Erb S, Stranden A, Kuijper EJ, Knetsch CW, et al. Transmissibility of Clostridium difficile Without Contact Isolation: Results From a Prospective Observational Study With 451 Patients. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017;64(4):393-400.
74. Feazel LM, Malhotra A, Perencevich EN, Kaboli P, Diekema DJ, Schweizer ML. Effect of antibiotic stewardship programmes on Clostridium difficile incidence: a systematic review and meta-analysis. J Antimicrob Chemother. 2014;69(7):1748-54.
75. Finn E, Andersson FL, Madin-Warburton M. Burden of Clostridioides difficile infection (CDI) - a systematic review of the epidemiology of primary and recurrent CDI. BMC Infect Dis. 2021;21(1):456.
76. Morgan DJ, Diekema DJ, Sepkowitz K, Perencevich EN. Adverse outcomes associated with Contact Precautions: a review of the literature. Am J Infect Control. 2009;37(2):85-93.
77. Fishbein SRS, Hink T, Reske KA, Cass C, Struttmann E, Iqbal ZH, et al. Randomized Controlled Trial of Oral Vancomycin Treatment in Clostridioides difficile-Colonized Patients. mSphere. 2021;6(1).
78. Horvat S, Rupnik M. Interactions Between Clostridioides difficile and Fecal Microbiota in in Vitro Batch Model: Growth, Sporulation, and Microbiota Changes. Front Microbiol. 2018;9:1633.
79. Scott AJ, Alexander JL, Merrifield CA, Cunningham D, Jobin C, Brown R, et al. International Cancer Microbiome Consortium consensus statement on the role of the human microbiome in carcinogenesis. Gut. 2019;68(9):1624-32.
80. Kostic AD, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: current status and the future ahead. Gastroenterology. 2014;146(6):1489-99.
81. Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. Clin J Gastroenterol. 2018;11(1):1-10.
82. Raskov H, Burcharth J, Pommergaard HC, Rosenberg J. Irritable bowel syndrome, the microbiota and the gut-brain axis. Gut Microbes. 2016;7(5):365-83.
83. Singer-Englar T, Barlow G, Mathur R. Obesity, diabetes, and the gut microbiome: an updated review. Expert Rev Gastroenterol Hepatol. 2019;13(1):3-15.
84. Collins SM, Surette M, Bercik P. The interplay between the intestinal microbiota and the brain. Nat Rev Microbiol. 2012;10(11):735-42.
85. Vendrik KEW, Ooijevaar RE, de Jong PRC, Laman JD, van Oosten BW, van Hilten JJ, et al. Fecal Microbiota Transplantation in Neurological Disorders. Front Cell Infect Microbiol. 2020;10:98.
86. Arthur JC, Jobin C. The complex interplay between inflammation, the microbiota and colorectal cancer. Gut Microbes. 2013;4(3):253-8.
87. Nooij S, Ducarmon QR, Laros JFJ, Zwittink RD, Norman JM, Smits WK, et al. Fecal Microbiota Transplantation Influences Procarcinogenic Escherichia coli in Recipient Recurrent Clostridioides difficile Patients. Gastroenterology. 2021;161(4):1218-28.e5.
88. Nichols RG, Peters JM, Patterson AD. Interplay Between the Host, the Human Microbiome, and Drug Metabolism. Hum Genomics. 2019;13(1):27.

89. Wilson BE, Routy B, Nagrial A, Chin VT. The effect of antibiotics on clinical outcomes in immune-checkpoint blockade: a systematic review and meta-analysis of observational studies. Cancer Immunol Immunother. 2020;69(3):343-54.

90. Derosa L, Hellmann MD, Spaziano M, Halpenny D, Fidelle M, Rizvi H, et al. Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. Ann Oncol. 2018;29(6):1437-44.

91. Pinato DJ, Howlett S, Ottaviani D, Urus H, Patel A, Mineo T, et al. Association of Prior Antibiotic Treatment With Survival and Response to Immune Checkpoint Inhibitor Therapy in Patients With Cancer. JAMA Oncol. 2019;5(12):1774-8.

92. Mohiuddin JJ, Chu B, Facciabene A, Poirier K, Wang X, Doucette A, et al. Association of Antibiotic Exposure With Survival and Toxicity in Patients With Melanoma Receiving Immunotherapy. J Natl Cancer Inst. 2021;113(2):162-70.

93. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science. 2018;359(6371):97-103.

94. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science. 2018;359(6371):91-7.

95. Daillère R, Routy B, Goubet AG, Cogdill A, Ferrere G, Alves-Costa Silva C, et al. Elucidating the gut microbiota composition and the bioactivity of immunostimulatory commensals for the optimization of immune checkpoint inhibitors. Oncoimmunology. 2020;9(1):1794423.

96. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med. 2013;368(5):407-15.

97. Cammarota G, Masucci L, Ianiro G, Bibbò S, Dinoi G, Costamagna G, et al. Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent Clostridium difficile infection. Aliment Pharmacol Ther. 2015;41(9):835-43.

98. Quraishi MN, Widlak M, Bhala N, Moore D, Price M, Sharma N, et al. Systematic review with meta-analysis: the efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory Clostridium difficile infection. Aliment Pharmacol Ther. 2017;46(5):479-93.

99. Baktash A, Terveer EM, Zwittink RD, Hornung BVH, Corver J, Kuijper EJ, et al. Mechanistic Insights in the Success of Fecal Microbiota Transplants for the Treatment of Clostridium difficile Infections. Front Microbiol. 2018;9:1242.

100. Suez J, Zmora N, Zilberman-Schapira G, Mor U, Dori-Bachash M, Bashiardes S, et al. Post-Antibiotic Gut Mucosal Microbiome Reconstitution Is Impaired by Probiotics and Improved by Autologous FMT. Cell. 2018;174(6):1406-23.e16.

101. Besselink MG, van Santvoort HC, Renooij W, de Smet MB, Boermeester MA, Fischer K, et al. Intestinal barrier dysfunction in a randomized trial of a specific probiotic composition in acute pancreatitis. Ann Surg. 2009;250(5):712-9.

102. Feuerstadt P, Louie TJ, Lashner B, Wang EEL, Diao L, Bryant JA, et al. SER-109, an Oral Microbiome Therapy for Recurrent Clostridioides difficile Infection. N Engl J Med. 2022;386(3):220-9.

103. Khanna S, Assi M, Lee C, Yoho D, Louie T, Knapple W, et al. Efficacy and Safety of RBX2660 in PUNCH CD3, a Phase III, Randomized, Double-Blind, Placebo-Controlled Trial with a Bayesian Primary Analysis for the Prevention of Recurrent Clostridioides difficile Infection. Drugs. 2022;82(15):1527-38.

104. Dsouza M, Menon R, Crossette E, Bhattarai SK, Schneider J, Kim YG, et al. Colonization of the live biotherapeutic product VE303 and modulation of the microbiota and metabolites in healthy volunteers. Cell Host Microbe. 2022;30(4):583-98.e8.

105. Fishbein SR, Robinson JI, Hink T, Reske KA, Newcomer EP, Burnham CD, et al. Multi-omics investigation of Clostridioides difficile-colonized patients reveals pathogen and commensal correlates of C. difficile pathogenesis. Elife. 2022;11.

106. Ooijevaar RE, Terveer EM, Verspaget HW, Kuijper EJ, Keller JJ. Clinical Application and Potential of Fecal Microbiota Transplantation. Annu Rev Med. 2019;70:335-51.