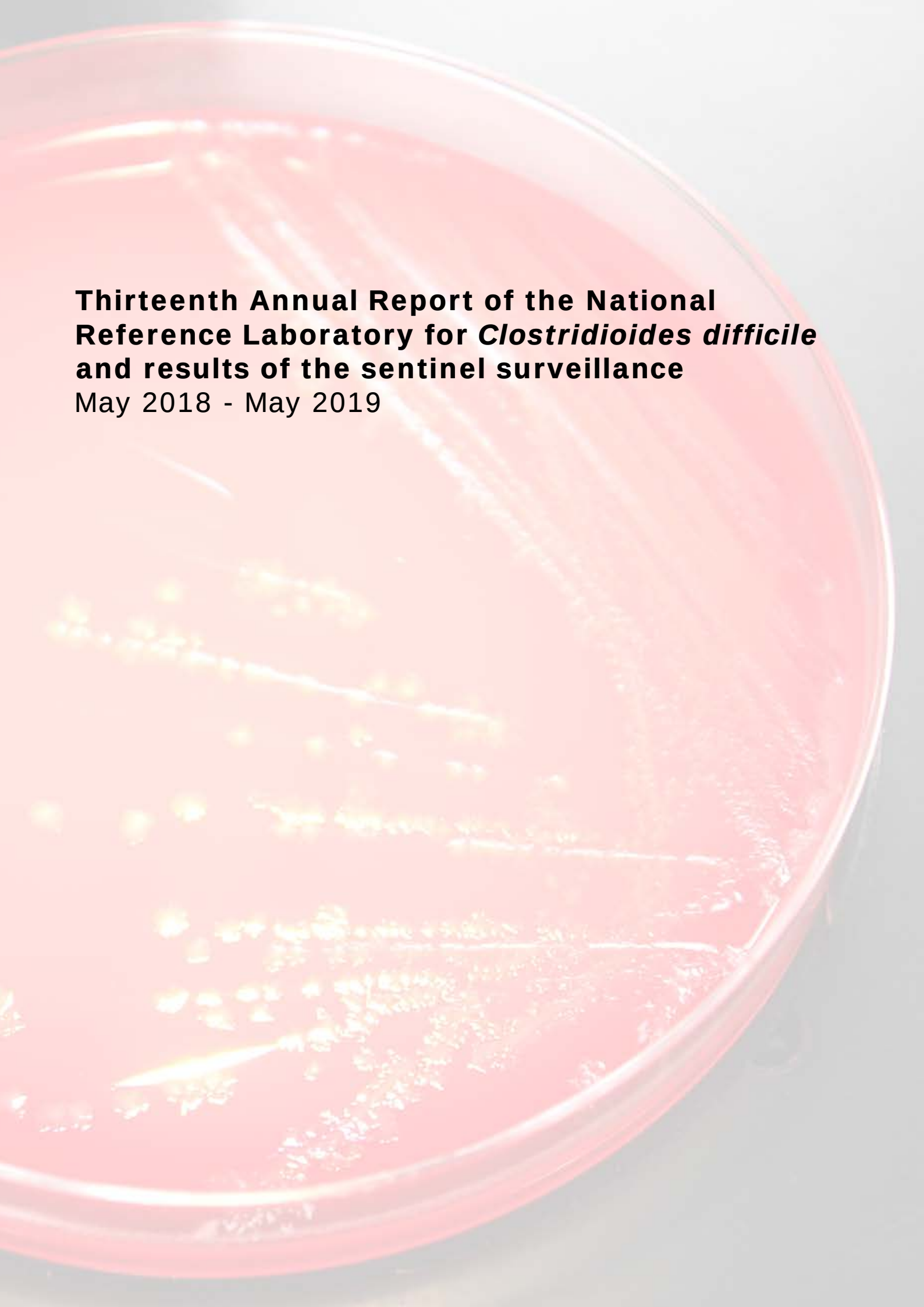

Thirteenth Annual Report of the National Reference Laboratory for *Clostridioides difficile* and results of the sentinel surveillance

May 2018 - May 2019

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Content

Introduction

Clostridioides difficile (C. difficile) is an anaerobic, spore-forming bacterium which can colonise the intestine of humans and animals. Pathogenic *C. difficile* strains can produce protein toxins (toxin A and/or B, and/or binary toxin) that disrupt the intestinal wall and thereby cause mild diarrhoea, severe colitis or a life-threatening toxic megacolon depending on host susceptibility and the virulence of the infecting strain.[1]

Diagnosis

The diagnosis of *C. difficile* infection (CDI) is most frequently based on clinical signs and symptoms in combination with laboratory tests. In 2016, a revision of the ESCMID guidelines on CDI diagnosis was published.[2] According to these guidelines the use of a two-step algorithm to diagnose CDI is recommended. These guidelines also stress the fact that a distinction between CDI patients and *C. difficile* carriers is not possible if only tests that detect the toxin-producing potential (i.e. toxin B PCR or toxigenic culture) are used instead of the detection of free toxins present in stools (i.e. by toxin A/B enzyme immunoassay). The ECDC surveillance protocol for CDI also recommends to use a two–step algorithm. Alternatives to laboratory diagnosis are endoscopy or histopathology to diagnose pseudomembranous colitis, though this diagnosis is not specific for CDI and is also associated with other enteropathogens or medication effects. Cultured isolates can be subtyped by PCR ribotyping. PCR ribotyping uses the PCR ribotype-dependent differences in profiles generated by PCR amplification of the intergenic spacer regions between the 23S and 16S rRNA genes.[3] The Reference Laboratory is currently able to recognise 273 different PCR ribotypes and is exploring application of whole genome sequencing for typing of *C. difficile*.

Transmission and infection control

Transmission of *C. difficile* within the hospital setting is common. However, the changing view is that *C. difficile* is not only transmitted by symptomatic CDI patients. Asymptomatic carriers can also introduce the bacterium into the hospital and spread it to other patients, although at a lower rate than symptomatic CDI patients.[4,5] Yet, standard infection control precautions focus on CDI patients only. The national WIP guideline (July 2011) recommends application of contact precautions in combination with hospital cleaning and disinfection[6], though many Dutch hospitals do not enforce the use of high concentrations of chloride due to occupational health issues. Antibiotic stewardship is another important factor in reducing CDI incidence.[7] At the moment, detecting and isolating *C. difficile* carriers is not generally recommended. Also, most hospitals stop contact precautions 48 hours after the last diarrhoeal symptoms, although it is known that CDI patients may shed spores for a prolonged amount of time.[8]

In 2018, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) study group for *C. difficile* (ESGCD) published an updated guideline on CDI prevention[9] using a systematic review of the literature. Screening for *C. difficile* to identify colonised/carrier patients or healthcare workers was not recommended by the study group. For hand-hygiene, it is recommended to switch from alcohol-based hand rub (AHR) to washing with soap and water in an outbreak setting, but not in an endemic situation. Contact precautions and the use of personal protective equipment (PPE), such as gloves and gowns/disposable aprons, are also advised to decrease transmission of *C. difficile*. Daily and terminal environmental sporicidal disinfection should be applied in rooms of patients with CDI to eradicate spores. To achieve a reduction of the CDI rates in hospitals, the study group recommends the restriction of antibiotic agents/classes and a reduction of the duration of antibiotic therapy. The study group concludes that education for health care workers, environmental service personnel, CDI patients and visitors on prevention of CDI is very useful.

Treatment of *C. difficile* infection

The first step in the management of CDI is to discontinue the inciting antibiotic, if possible. Antibiotic treatment of CDI (with either metronidazole, vancomycin or fidaxomicin) is tailored by severity of disease and also differs for an initial episode, single recurrence or multiple recurrences.[10] In February 2018, the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA) published Clinical Practice Guidelines for CDI, which recommend vancomycin or fidaxomicin over metronidazole as treatment for an initial episode or recurrent CDI.[11] Similarly, a critical review from a study group in the Netherlands also concluded that vancomycin is preferred as

first agent of choice.[12] New ESCMID guidelines on the treatment of CDI are currently being compiled and will be completed in 2020.
Despite antibiotic therapy, CDI recurrence is common. Faecal microbiota transplantation is proven to be very effective as treatment for recurrent CDI, likely by restoring the healthy gut microbiota.[13] Due to the high costs and time-consuming nature of donor screening, faecal microbiota transplantation was earlier frequently not offered despite an indication for it. To overcome these problems, the National Donor Faeces Bank (NDFB) was set up at Leiden University Medical Centre in 2016 (http://www.ndfb.nl/). The aim of the NDFB is to make transplantation of carefully screened donor faeces easily available for treatment of patients with multiple relapsing CDI.[14] Donors are healthy volunteers who are screened according to a standardised protocol including questionnaires and microbiological investigations of serum and faeces. Stool preparations of these healthy donors are stored at the biobank in the LUMC. These ready-to-use frozen donor faeces suspensions can be ordered by treating physicians of patients with recurrent or severe CDI (info@ndfb.nl). Patients can receive the faecal microbiota transplantation at their local hospital. A total of n=136 faecal microbiota transplantations for recurrent or severe CDI with a faeces suspension from the NDFB were performed in the period May 2016-May 2019 with a cure rate of 90%.
Recently, bezlotoxumab (humanised monoclonal antibodies against *C. difficile* toxin B) has been tested in a clinical setting to prevent recurrent CDI.[15] More evidence is becoming available that suggests that bezlotoxumab may be an alternative option for the prevention of recurrent CDI, mainly in patient with risk factors for recurrence.[16,17] However, no studies have compared the efficacy of FMT and bezlotoxumab.

Epidemiology
Before 2005, CDI outbreaks were rarely reported in the Netherlands. In 2005, the *C. difficile* ribotype 027 strain (or NAP1/REA BI strain) was for the first time detected[18] and rapidly spread within Netherlands while causing major outbreaks.[19,20] Retrospectively, the rapid spread of the ribotype 027 strain across Northern-America and Europe has been attributed to its high level of fluoroquinolone resistance.[21] A study by *Collins et al* suggests that the rapid spread might also be attributed to a different trehalose metabolism in ribotype 027 strains, which causes the ability to metabolise low concentrations of trehalose. The implementation of trehalose as a food additive into the human diet, shortly before the emergence of ribotype 027, might have stimulated the spread of ribotype 027.[22] CDI cases due to ribotype 027 were associated with unfavourable patient outcomes such as severe disease, mortality and recurrent CDI in comparison to other ribotypes[19,23], which may reflect type-specific host susceptibility and/or an increased virulence of the strain.[24] Since mid-2006, the occurrence of ribotype 027 in the Netherlands has decreased significantly.[25] The CDI incidence rate has stabilised at 3 CDI cases per 10,000 patient-days.[26] Interestingly, more ribotype 027-like strains are beginning to emerge, like ribotype 036, 198 and 181. They are mainly observed in Eastern-Europe. In 2019, four isolates with ribotype 198 were found in one Dutch hospital, while this ribotype has only been observed once before in the Netherlands by the Dutch Reference laboratory.

Surveillance and ad hoc typing
The Centre for Infectious Disease Control (CIb) of the National Institute for Public Health and the Environment (RIVM) started a National Reference Laboratory for *C. difficile* at the Leiden University Medical Centre soon after recognition of *C. difficile* ribotype 027 outbreaks in 2005. Since then, this laboratory has offered ad hoc typing service for all microbiology laboratories in the Netherlands for typing of *C. difficile* isolates of patients with severe disease, or isolates from a suspected outbreak. Additionally, the National Reference Laboratory initiated a sentinel surveillance programme in May 2009 to monitor the incidence of CDI in an endemic situation. Furthermore, the programme aims to monitor (new) emerging strains of *C. difficile*.
In the period of May 2018 until May 2019 twenty-four acute care hospitals participated in the sentinel surveillance programme voluntarily. Each year, results are reported on the website of the National Institute for Public Health and the Environment (RIVM).[26] This current report is the thirteenth annual report that provides an overview of the two types of surveillance conducted in the Netherlands, describing the situation in the Netherlands between May 1st 2018 and May 1st 2019.

The Netherlands is also participating in the European-wide CDI surveillance which is led by ECDC. For this, the data of all participating hospitals of the Dutch sentinel surveillance is sent to ECDC once every year. The protocol for this European surveillance program is available at:

https://ecdc.europa.eu/en/publications-data/european-surveillance-clostridium-difficile-infections-surveillance-protocol-1.

Aims and procedures of the sentinel surveillance

The aims of the national sentinel surveillance of *C. difficile* infections are:

1. To obtain continuous incidence rates of patients with CDI in participating hospitals in the Netherlands.
2. To identify and characterise new circulating PCR ribotypes.
3. To correlate newly found circulating PCR ribotypes with changes of epidemiology and clinical syndromes of CDI.

Patient inclusion

Hospitals participating in the sentinel surveillance are requested to include in the surveillance all hospitalised patients >2 years with clinical signs or symptoms of CDI in combination with a positive test for *C. difficile* toxins or toxigenic *C. difficile*. Patients are tested on their physicians' request or without a specific request, for instance if they are admitted to the hospital for three days or more and their unformed stool is submitted to the laboratory (the "three day rule"). The assay or algorithm that is used to diagnose CDI, is chosen by the local laboratory. Laboratories that culture *C. difficile* (n=11) send strains to the laboratory of the Leiden University Medical Centre. Other laboratories (n=10) send faecal samples. Some laboratories (n=3) send faecal samples or strains.

Collection of patient data

The OSIRIS system is used to complete a web-based questionnaire for each included patient. This questionnaire contains questions involving patient's gender, age, location of onset of the infection, symptoms of the infection and antibiotic use. Furthermore, the outcome after 30 days is requested. The definitions applied in this questionnaire are based on those proposed by the ECDC and the CDC.[27,28] In the OSIRIS system, the results of the PCR ribotyping (completed by the reference laboratory) are linked to the data of the questionnaire. Analysis of clinical and demographic characteristics in combination with the results of PCR ribotyping can be performed by all participating individual laboratories for their own data.

Microbiological reports

All faecal samples are cultured and *C. difficile* isolates are characterised (see next chapter) at the laboratory of the Leiden University Medical Centre. In case PCR ribotype 027 (or a 027-like ribotype) is found, the local microbiologist is directly informed by telephone and asked if there is a need for additional information or advice. If there is suspicion of an outbreak, laboratories will also be contacted as soon as possible by telephone and e-mail. Once a week, microbiological results are sent by e-mail to the submitting microbiologist, infection control practitioners, and, when an outbreak is suspected or ribotype 027 isolated, also to CIb. The results are also reported in OSIRIS. All submitting laboratories also receive the official report by regular post. Once a year, an overview of the results of the sentinel surveillance is provided to the participating hospitals by a national report and a special meeting organised by the LUMC and RIVM.

Incidence rates and outbreaks

The last data-extraction for this annual report was performed on August 16th 2019. To calculate incidence rates, we requested the participating hospitals to register their monthly number of admissions and number of patient-days. If no data were available, the data from the previous year were used as denominator. Incidence rates are estimated by the number of CDI patients per 10,000 patient-days. These numbers might be a slight underestimation, as children below 2 years old are excluded from the surveillance but are included in the denominator data for feasibility. The 95% confidence intervals for incidence rates were calculated by Byar's Approximation.
A suspected outbreak was defined if >2 isolates of the same type were found less than 7 days apart in one hospital, either with onset of symptoms on the same department, or accompanied with an increased CDI monthly incidence within the hospital.
Statistical analysis were performed using Excel and STATA/SE for Windows software package, version 15.1. Maps were created through FreeVectorMaps.com.

Aims and procedures of the ad hoc typing

The aims of the ad hoc typing are:

1. To provide medical microbiological laboratories not participating in the sentinel surveillance the opportunity to have *C. difficile* strains isolated and typed in case of suspected outbreaks in hospitals or other health care facilities.
2. To isolate *C. difficile* for further typing from faecal samples of patients with CDI sent to the reference laboratory by laboratories that do not culture *C. difficile*.
3. To characterise isolated *C. difficile* strains by PCR ribotyping, presence of genes *tcd*A and *tcd*B, presence of binary toxin genes and the presence of deletions in *tcd*C.
4. To investigate the relatedness of the isolates by Multiple-Locus Variable number tandem repeat Analysis (MLVA) or by whole genome MultiLocus Sequence Typing (wg MLST).[29,30]
5. To report the results of the investigation to CIb and to medical microbiologists who submitted the samples from severe CDI diseases or outbreaks.
6. To obtain demographical data and clinical information of the patients with microbiological proven CDI.

C. difficile isolation

Isolation of *C. difficile* from faecal samples at the Reference laboratory is performed on *C. difficile* selective agar supplemented with cefoxitin, amphotericin B and cycloserine (CLO-medium; BioMérieux), with and without ethanol shock pre-treatment. After incubation in an anaerobic environment at 37 °C for 48h, colonies of Gram-positive rods with subterminal spores are tested for the presence of the glutamate dehydrogenase gene by an in-house PCR.

C. difficile confirmation

All isolates are genetically identified as *C. difficile* by an in-house PCR for the presence of the *gluD* gene, encoding the glutamate dehydrogenase (GDH) specific for *C. difficile*.[28] All *C. difficile* strains are further investigated by PCR-ribotyping.[3] The presence of *tcdA*, *tcdB* and binary toxin genes is investigated by multiplex PCR on request.[31] Deletions in *tcdC* can be determined by PCR using in-house designed primers.

C. difficile Reference Library

The Reference Laboratory added 10 new ribotypes to the Reference Library in the prior year, and is now able to recognise 273 different PCR ribotypes. If an unknown ribotype is isolated more than 5 times, the electronic capillary PCR ribotyping profiles are send to the Department of Microbiology, Leeds Teaching Hospitals NHS Trust, Leeds (dr. Warren Fawley, prof. Mark Wilcox), to assign a (new) ribotype.

Microbiological reports

Results of microbiological analysis are sent by e-mail to the submitting microbiologist and to CIb. When PCR ribotype 027 (or a 027-like ribotype) is found, the laboratories are also informed by telephone and are offered to contact the LUMC or CIb for additional information and advice. Submitting laboratories also receive an official report by regular post.

Collection of patient data

A standardised questionnaire is used to obtain information on patient's age and gender, the ward where CDI was acquired, clinical data, risk factors, antibiotic treatment in the month preceding a positive test and treatment outcomes. The definitions applied in this questionnaire are based on those proposed by the ECDC and the CDC.[27,28] Co-morbidity is defined according to the ICD-10 classification. The questionnaires are sent by e-mail to the submitting laboratories when faecal samples or isolates are received.

Results of the sentinel surveillance

Participating hospitals

This section describes the results of the 24 participating hospitals of the sentinel surveillance programme in the period May 2018 - May 2019. Both university hospitals (n=5) and primary or secondary care hospitals (n=19) were included, distributed all over the Netherlands. The geographical location of the participating hospitals is displayed in Figure 1.

Figure 1. Participating hospitals of the sentinel surveillance between May 2018 and May 2019. University hospitals are depicted in orange, primary/secondary care hospitals are depicted in blue

Diagnostic testing

The diagnostic tests used by the participating hospitals to diagnose CDI are depicted in Table 3 and Figure 2. By May 2019, 12/24 hospitals (52.1%) used an ESCMID recommended algorithm (algorithms A and B), which is more than last year (40.9%). Another 10 hospitals (43.5%) used a stand-alone nucleic acid amplification test (NAAT) which is either a PCR or a loop-mediated isothermal amplification (LAMP) assay to detect toxin A and/or B genes. Three of the 10 hospitals relying on NAAT performed culture on NAAT-positive samples for confirmation and to have the isolates available for typing. One hospital used an enzyme immunoassay for toxins A/B (Tox A/B EIA) as a stand-alone test. Six of the 24 hospitals (25.0%) tested all submitted unformed stool samples from hospitalised patients 2 years or older for CDI. Nine out of 24 hospitals (39.1%) tested unformed stool samples from patients admitted for at least 3 days (the so-called “three day rule”) or with a specific request for CDI testing. Another 6 hospitals (26.1%) tested samples with a request for CDI testing only. In most hospitals, restrictions applied for CDI testing of stool samples from young children (<2 years) (Figure 3). The mean percentage of *C. difficile* positive patients among all patients tested was 7.3% (range 2.6-11.4%; Table 3).

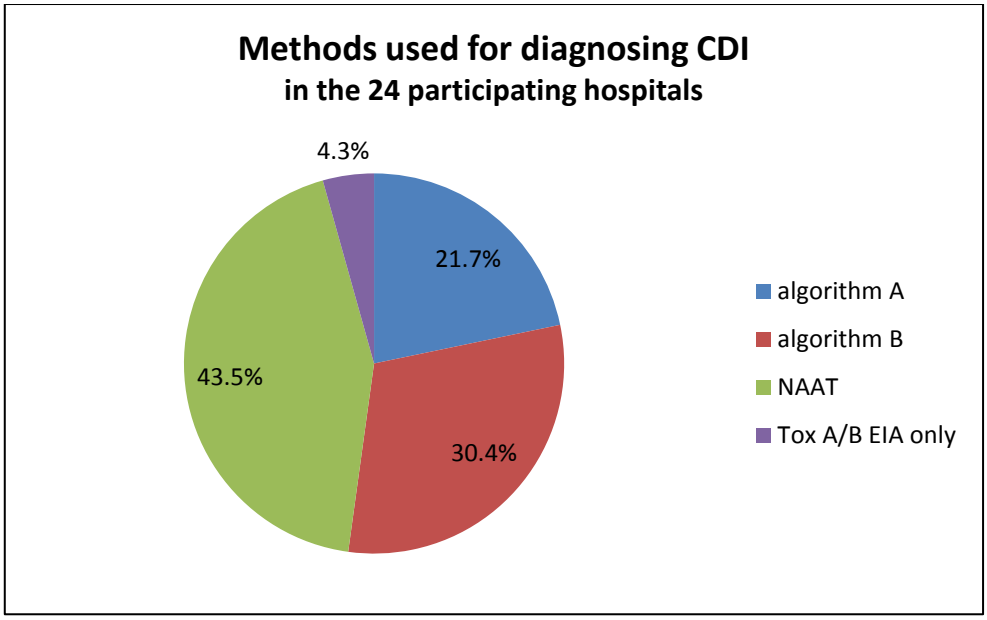


Figure 2. Laboratory methods used for diagnosing CDI in the 24 hospitals participating in the sentinel surveillance program. Algorithm A (NAAT or GDH EIA - Tox A/B EIA) and B (GDH & Tox A/B EIA, and in some hospitals confirmation with NAAT/toxigenic culture) are ESCMID-recommended methods, all the others are non-recommended methods.

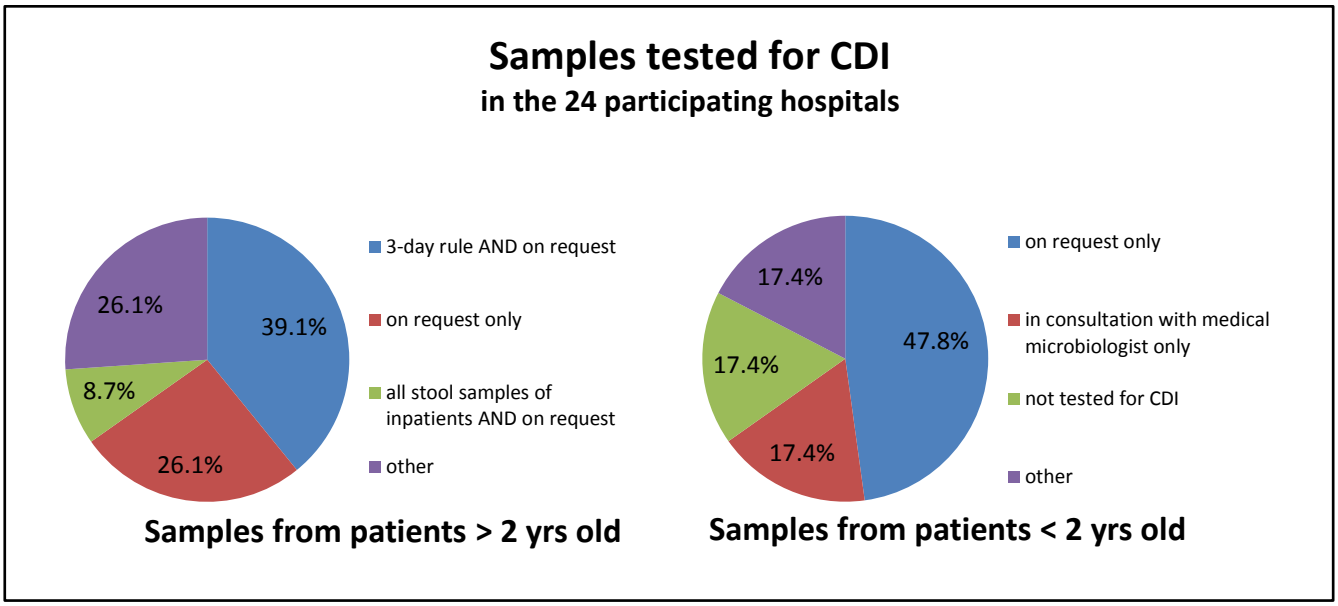


Figure 3. Samples tested for CDI in the 24 hospitals participating in the sentinel surveillance. Selection criteria for samples from patients >2 years are shown on the left, selection criteria for samples from patients <2 years are shown on the right.

Incidence in participating hospitals

The numbers of CDI per 10,000 patient-days per hospital are shown in Table 3, and are compared to the incidence rate of the preceding year. The mean incidence was 3.17 CDI cases per 10,000 patient-days (varying from 0.95 to 5.88 CDI cases per 10,000 patient-days), comparable to the incidence of 2.90 that was reported in 2017-2018.[26] For hospitals that submitted data on monthly patient-days or hospitals of which monthly patient-days of the previous year were available (22 hospitals), the overall monthly CDI incidence rates were calculated over the year (see Figure 4).

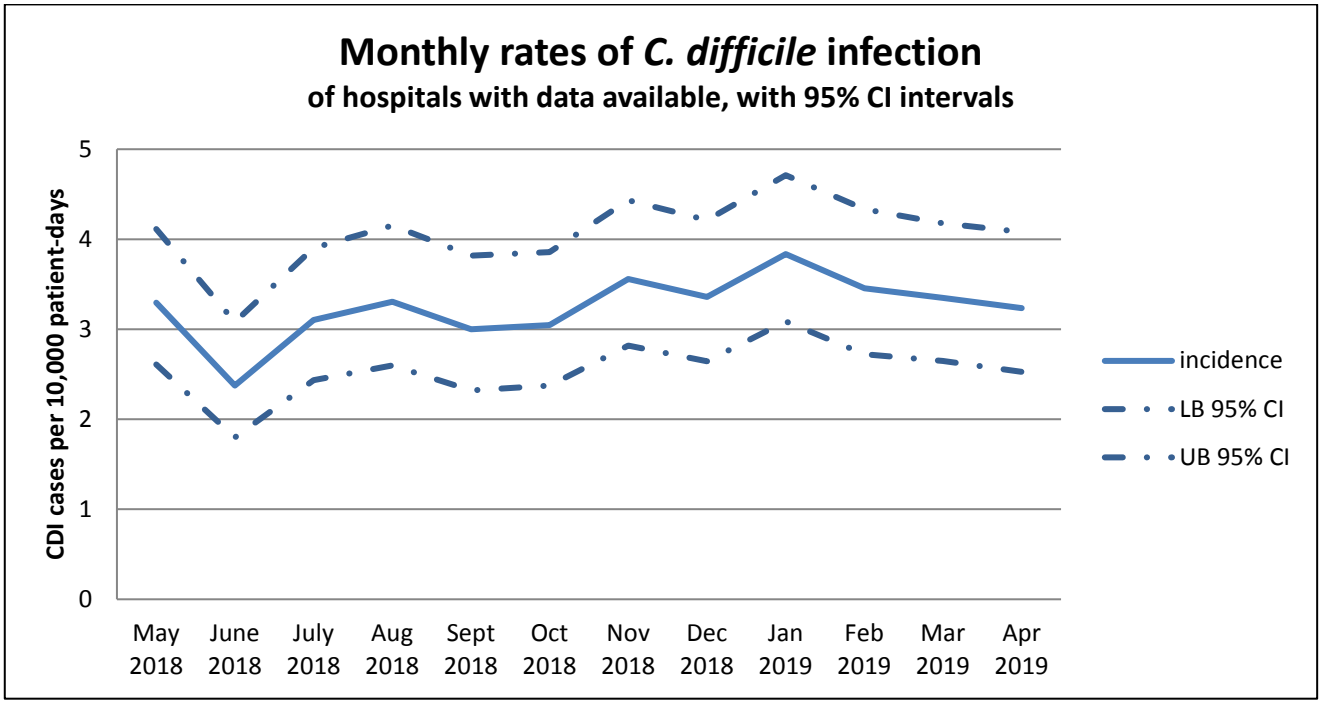


Figure 4. Monthly rates of CDI (cases per 10,000 patient-days) in 22 of the participating hospitals. LB 95% CI; lower bound 95% confidence interval, UB 95% CI; upper bound 95% confidence interval. When PD data of this year was not available per month, data of the previous year was used.

Submitted strains for PCR ribotyping

Of 900 CDI patients included in sentinel surveillance between May 1st 2018 and May 1st 2019, 718 *C. difficile* isolates could be PCR ribotyped and linked to the clinical data (79.8%). The most important reasons for missing data were the inability to culture *C. difficile* at the local laboratory, no registration of the patient in OSIRIS, not sending the isolates or faeces to the National Reference Laboratory (n=113) or the inability to type *C. difficile* at the National Reference laboratory (culture negative or other *C.* species; n=69).

Circulating PCR ribotypes

Similar as the previous year, ribotype 014/020 was the most frequently isolated ribotype.
This year ribotype 078/126 was the second most frequently isolated ribotype, in contrast to last year when this was ribotype 002.
Ribotype 014/020 (014 and 020 are indistinguishable by conventional PCR ribotyping) was isolated in 140 of the 718 samples (19.5%; 95% CI 16.6-22.4). The closely related ribotypes 078 and 126 were found in 84 isolates (11.7%; 95% CI 9.3-14.1). Ribotype 002 was found in 69 isolates (9.6%; 95% CI 7.5-11.8), ribotype 005 in 42 isolates (5.8%; 95% CI 4.1-7.6), and ribotype 001 in 40 isolates (5.6%; 95% CI 3.9-7.2). Four isolates were identified as ribotype 027 (0.6%; 95% CI 0.0-1.1). Of 34 isolates (4.7%; 95% CI 3.2-6.3) the PCR ribotype pattern was not recognised in our database. Of these isolates, 1 pair of unknown ribotypes was exactly the same.
The results stratified per participating centre are displayed in Table 4. A pie-chart of the five most common ribotypes and the hypervirulent ribotypes of patients included in the sentinel surveillance is illustrated in Figure 8.

Changes in circulating PCR ribotypes

In Figure 5, the proportions of the 5 most common ribotypes are shown in time.
Ribotype 014/020 had a proportion of 11.9% (95% CI 9.1-14.7) at the start of the sentinel surveillance in 2009-2010 and a proportion of 19.5% (95% CI 16.6-22.4) in 2018-2019. The proportion of ribotype 078/126 was comparable to the previous year (2018-2019 95% CI 9.3-14.1, 2017-2018 95% CI 7.7-12.2). The proportion of ribotype 002 was also not different compared to the previous year (2018-2019 95% CI 7.5-11.8, 2017-2018 95% CI 9.4-14.3). The proportion of RT001 was 26.5% (95% CI 22.7-30.3) at the start of the surveillance in 2009-2010 and 5.6% (95% CI 3.9-7.2) in 2018-2019. In 2016-2017, there was an outbreak of ribotype 001 with an increased proportion of ribotype 001. The proportion of ribotype 001 has decreased compared to 2016-2017 and is now comparable to the proportion in 2015-2016 (2018-2019 95% CI 3.9-7.2, 2016-2017 95% CI 8.2-12.2, 2015-2016 95% CI 2.1-4.7).
The proportion of ribotype 027 was not different from last year (2018-2019 95% CI 0.0-1.1, 2017-2018 95% CI 0.4-2.0). The proportion remained lower than in some of the previous years (2009-2010 95% CI 2.5-6.0, 2010-2011 95% CI 1.1-3.6, 2011-2012 95% CI 1.1-3.4, 2012-2013 95% CI 2.0-4.8, 2013-2014 95% CI 1.9-4.6, see Figure 6). Ribotype 027 was found in 4 individual cases in 3 hospitals (3/24; 12.5%).

(Suspected) outbreaks in participating hospitals

In the period between May 1st 2018 and May 1st 2019, no outbreaks of *C. difficile* in hospitals participating in the sentinel surveillance were reported to the National Reference Laboratory.

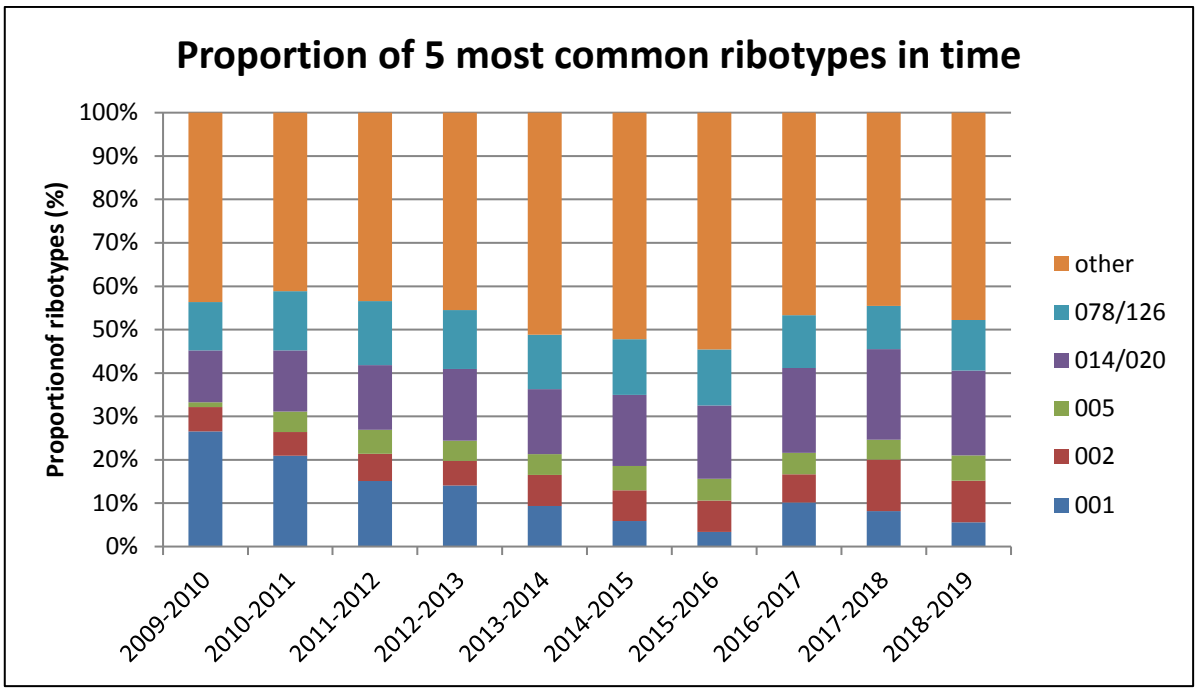


Figure 5. Proportions of the 5 most common ribotypes in time in sentinel surveillance samples.

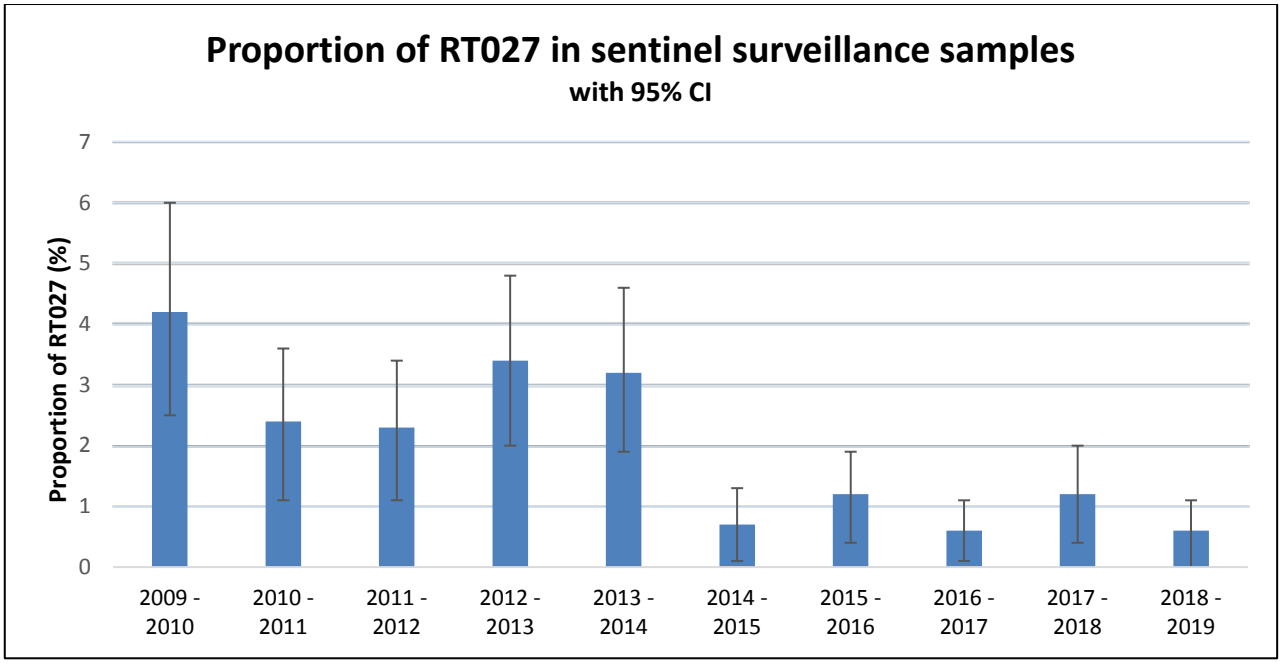


Figure 6. Proportion of 027 in sentinel surveillance samples. CI; confidence interval.

Demographical and clinical data

Demographical and clinical characteristics were collected from 900 patients included in the sentinel surveillance (Table 1). The mean age was 66.5 years (95% CI 65.3-67.7). Of all patients, 2.3% (n=21) was younger than 18 years old and 61.0% (n=549) was older than 65 years old. Furthermore, 45.8% of the patients had a community-onset of symptoms and 54.2% a healthcare facility-onset of symptoms. A total of 141 patients (16.1%) had severe CDI, defined as bloody diarrhoea and/or diarrhoea with hypovolaemia or hypoalbuminaemia (<20g/L) and/or with fever (T >38.0 °C) and leucocytosis (WBC count >15x10^9/l), and/or with pseudomembranous colitis. After 30 days, the outcome and course of the disease was known for 812 patients. In total 731 patients (90.0%) had an uncomplicated course of their CDI infection. On the other hand, 2 patients (0.2%) were admitted to the ICU as a consequence of CDI, 3 patients (0.4%) needed surgery as a consequence of CDI and 76 patients with CDI (9.3%) died. Nine deaths (1.1%) were due or contributable to CDI.

Comparison to previous years

Data from the sentinel surveillance were compared to surveillance data from previous years (Table 2). The CDI incidence was similar as the incidence in previous years. The proportion of patients with severe CDI and proportion of patients with a complicated course of CDI were comparable to last year. However, in figure 7, a difference in severity of CDI is observed between the start of the surveillance in 2009-2010 with 27.9% (95% CI 23.9-31.9), and 2018-2019 with 16.1% (95% CDI 13.6-19.5). This may be explained by the decrease in the proportion of ribotype 027.

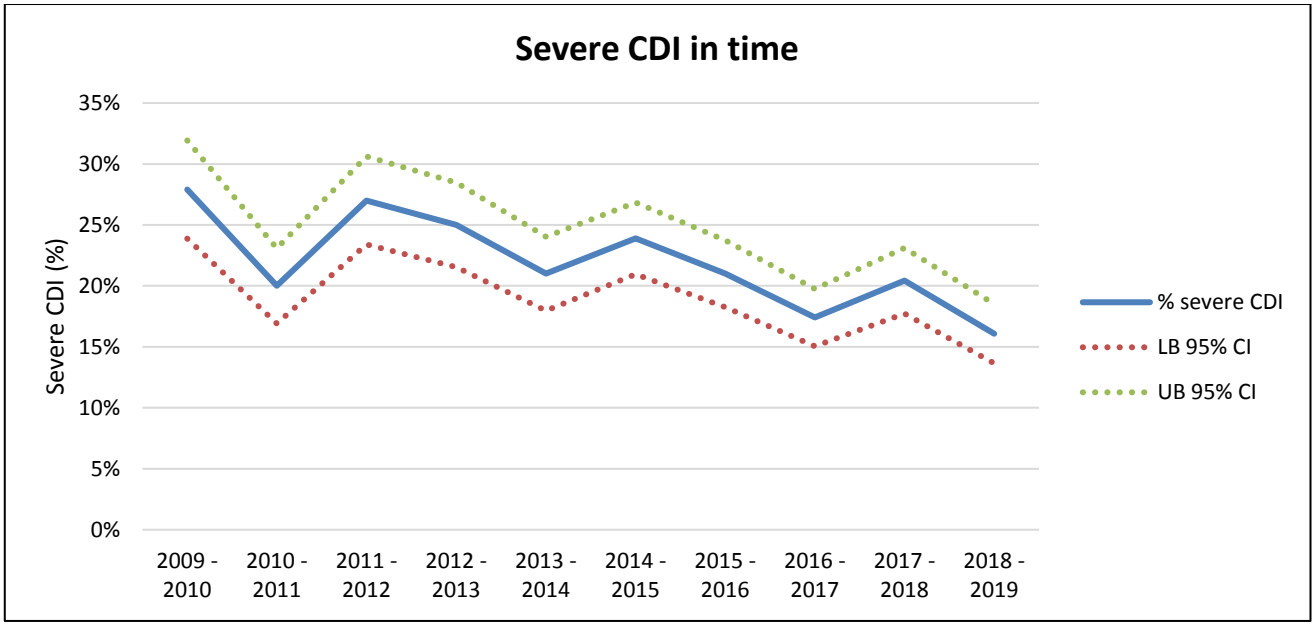


Figure 7. Proportion of patients with severe CDI in sentinel surveillance samples in time. LB 95% CI; lower bound 95% confidence interval, UB 95% CI; upper bound 95% confidence interval.

Furthermore, CDI-related mortality was 3.1% (95% CI 1.9-4.3) in 2017-2018 and decreased to 1.1% (95% CI 0.4-1.8) in 2018-2019. Overall mortality in CDI patients was comparable to the previous year.

The proportion of community-onset CDI cases was 37% (95% CI 32.9-41.1) at the start of the surveillance in 2009-2010, which was significantly lower than the proportion in 2018-2019, which was 46% (95% CI 42.7-49.3).

Table 1. Clinical characteristics and outcome of patients participating in the sentinel surveillance (n=900)

Patient characteristics and outcome	n / n[a]	%
Gender female	462/900	51.3%
Location of onset CDI		
hospital	435/897	48.5%
at home	411/897	45.8%
nursing home	29/897	3.2%
other health-care facility	22/897	2.5%
Hospital department		
Internal Medicine[1]	210/505	41.6%
Surgery[2]	69/505	13.7%
Lung diseases and TB	42/505	8.3%
Geriatrics	23/505	4.6%
Gastroenterology	46/505	9.1%
Cardiology	22/505	4.4%
ICU	16/505	3.2%
Neurology	14/505	2.8%
Pediatrics	7/505	1.4%
Other or unknown	25/505	5.0%
Antibiotics prior to CDI[3]	534/817	65.4%
Recurrence	179/650	27.5%
Severe CDI[3]	141/877	16.1%
Pseudomembranous colitis	20/877	2.3%
Hypovolaemia or hypo-albuminaemia	68/877	7.8%
Bloody diarrhoea	39/877	4.4%
Fever and leucocytosis	55/877	6.3%
Outcome[3,4]		
Uncomplicated	731/812	90.0%
Surgery needed	3/812	0.4%
ICU admission needed due to CDI	2/812	0.2%
Death, contributable to CDI	9/812	1.1%
Death, unrelated to CDI	65/812	8.0%
Death, cause unknown	2/812	0.2%

[1] Internal medicine including haematology, oncology, nephrology
[2] Surgery including orthopaedics and traumatology
[3] Missing data of hospital E
[4] Hospital R: outcome after 3 days instead of after 30 days

Table 2. Data from the sentinel surveillance for the period May 2018 - May 2019 compared to the data from preceding years.

Surveillance period (May-May)	2009-2010	2010-2011	2011-2012	2012-2013	2013-2014	2014-2015	2015-2016	2016-2017	2017-2018	2018-2019
Incidence										
Per 10,000 patient-days	2.7	2.8	2.9	2.9	2.9	3.0	3.1	3.0	2.9	3.2
Location of onset										
Within healthcare facility	63%	73%	69%	63%	64%	59%	58%	59%	55%	54%
At home	37%	27%	31%	37%	36%	41%	42%	41%	45%	46%
Course and outcome[1,2]										
Severe CDI	28%	20%	27%	25%	21%	24%	21%	17%	20%	16%
Uncomplicated course	66%	86%	87%	88%	87%	86%	89%	87%	87%	90%
Deaths contributable to CDI	4%	3%	4%	2%	3%	4%	2%	2%	3%	1%
PCR ribotype 027										
Prevalence	4.2%	2.4%	2.3%	3.4%	3.2%	0.7%	1.2%	0.6%	1.2%	0.6%
N reported 027 outbreaks-sentinel surveillance	1	1	0	1	0	0	0	0	0	0
N reported 027 outbreaks-ad hoc typing	2	2	1	2	5	1	0	1	0	0

[1] 2018-2019: Missing data of hospital E

[2] 2018-2019: Hospital R: outcome after 3 days instead of after 30 days

Table 3. Number of patients included in the sentinel surveillance per hospital, and incidence data. Period: May 1st 2018 – May 1st 2019. The diagnostic test or algorithm used to diagnose CDI is shown per hospital. The incidence per 10,000 patient-days is compared to the results of the previous annual report, demonstrated as an incidence difference.

Hospital	Diagnostic test(s)	Sample selection	% Positive	Monthly PD	Incidence per 10,000 PD 2018-2019	Incidence per 10,000 PD 2017-2018	Incidence difference
A*	algorithm B	on request only	6.0% (59/981)	15041	1.77	0.65	1.12
B	algorithm B	all unformed stool samples AND on request	5.9% (11/188)	3021	2.65	1.17	1.48
C	algorithm B	3-day rule AND on request	9.4% (39/417)	5343	2.96	1.40	1.57
D**	toxin A/B EIA	watery stool samples AND in admitted patients also mushy stool	3.7% (71/1933)	7671	4.13	1.85	2.28
E***	algorithm A	3-day rule AND on request	2.6% (8/304)	6230	2.54	1.87	0.67
F****	algorithm B	other criteria[2]	5.0% (30/599)	5488	1.21	2.13	-0.91
G	NAAT	3-day rule AND on request	11.1% (62/559)	10682	1.64	2.19	-0.55
H	algorithm A	on request AND all stool samples of inpatients	7.5% (138/1830)	14684	4.09	2.27	1.81
I	algorithm A	3-day rule AND on request	3.0% (68/2276)	9780	2.47	2.29	0.18
J	NAAT	on request AND all stool samples of inpatients	8.1% (51/626)	10564	0.95	2.41	-1.46
K	algorithm A	on request only	7.2% (49/678)	5949	2.80	2.98	-0.18
L	NAAT	on request only	7.2% (157/2167)	14364	2.84	3.06	-0.21
M	NAAT	on request only	9.8% (89/905)	11739	3.76	3.33	0.43
N	NAAT and culture	on request only	6.3% (269/4254)	13576	2.64	3.45	-0.81
O	NAAT	3-day rule AND on request	5.6% (48/862)	10867	3.53	3.52	0.01
P	algorithm B	3-day rule AND on request	6.2% (56/910)	10953	3.65	3.64	0.01
Q	NAAT	3-day rule AND on request	6.3% (97/1534)	13064	3.32	3.83	-0.52
R	NAAT[1]	on request only	11.4% (213/1873)	15053	3.43	3.88	-0.45
S***	algorithm B	on request AND if unformed only	9.8% (72/738)	8281	2.72	3.92	-1.21
T*****	NAAT and culture	on request AND on indication (unspecified)	7.9% (169/2135)	14605	5.88	3.99	1.88
U	algorithm B	3-day rule AND on request	10.3% (101/978)	6428	4.41	4.93	-0.52
V	NAAT and culture[1]	other criteria[3]	6.3% (300/4713)	18045	3.83	5.08	-1.25
W	NA	NA	NA	NA	NA	NA	NA
X	algorithm A	3-day rule AND on request	10.4% (20/192)	8436	5.63	NA	NA
			7.3%		**3.17**	**2.90**	**0.27**

NA=not available; PD=patient-days; NAAT=Nucleic Acid Amplification Test; EIA= enzyme immunoassay
algorithm A: NAAT or GDH EIA- Tox A/B EIA (*ESCMID recommended)*
algorithm B: GDH & Tox A/B EIA (and in some hospitals confirmation with NAAT/TC) *(ESCMID recommended)*
algorithm C: Tox A/B EIA - GDH EIA
* data % positive from 01-01-2018 untill 31-12-2018
** data % positive and PD of the prior year (data of this year not available)
*** data of the prior year (data of this year not available), except for number of cases;
**** PD from previous year
***** Excluding rehabilitation and psychiatry and neonatology

[1] Except in the weekend: then GDH and toxin A/B EIA is performed, and in some hospitals confirmation of these with NAAT/TC on the first following working day
[2] All unformed stool samples from inpatients and samples from immunocompromised patients, from patients with acute diarrhoea, during increased CDI incidence or on request
[3] on request AND all unformed stool samples of in- and outpatients
[4] General sample selection criteria apply also to children ≥1 and <2 years old. Children<1 year old: not tested for CDI
[5] When requested, physicians receive a message implying it is not useful in children<2 years old, but in case wanted, in consultation with medical microbiologist

Table 4. The two most frequently found PCR ribotypes per hospital, isolated amongst patients that were included in the sentinel surveillance. Period: 1st 2018 – May 1st 2019. Ribotype 014/020 are indistinguishable by conventional ribotyping, and ribotype 078/126 can be hardly discriminated.

Hospital	CDI Samples		Sample	*C. difficile isolated*		Most common type			2nd most common type		
	N	%	type	N	%		N	%		N	%
A	32	3.6%	Isolates	30	93.8%	002	7	23.3%	014/020	3	10.0%
B[1]	4	0.4%	Isolates or faeces	4	100.0%	several[5]	all n=1	25.0% each	-	-	-
C	19	2.1%	Faeces	16	84.2%	078/126	4	25.0%	014/020	2	12.5%
D	38	4.2%	Faeces	29	76.3%	014/020	4	13.8%	002, 023	both n=3	10.3% each
E	19	2.1%	Isolates	9	47.4%	014/020	3	33.3%	078/126	2	22.2%
F[2]	8	0.9%	Isolates or faeces	2	25.0%	014/020, 124	both n=1	50.0% each	-	-	-
G	21	2.3%	Isolates	17	81.0%	014/020, 078/126	both n=4	23.5% each	001	2	11.8%
H	72	8.0%	Faeces	68	94.4%	014/020	17	25.0%	002	14	20.6%
I	29	3.2%	Isolates	25	86.2%	005	5	20.0%	002, 014/020	both n=4	16.0% each
J[3]	4	0.4%	Faeces	4	100.0%	several[6]	all n=1	25.0% each	-	-	-
K	20	2.2%	Isolates	14	70.0%	002	3	21.4%	several[7]	all n=2	14.3% each
L	49	5.4%	Faeces	38	77.6%	078/126	6	15.8%	002, 014/020	both n=4	10.5% each
M	53	5.9%	Isolates or faeces	37	69.8%	014/020	7	18.9%	015	4	10.8%
N	43	4.8%	Isolates	41	95.3%	014/020	11	26.8%	several[8]	all n=3	7.3% each
O	46	5.1%	Isolates	38	82.6%	014/020	8	21.1%	several[9]	all n=4	10.5% each
P	48	5.3%	Faeces	37	77.1%	078/126	6	16.2%	002	5	13.5%
Q	52	5.8%	Isolates	36	69.2%	014/020	12	33.3%	076	3	8.3%
R	62	6.9%	Faeces	51	82.3%	014/020	12	23.5%	001	10	19.6%
S	27	3.0%	Faeces	22	81.5%	014/020	5	22.7%	015	3	13.6%
T	103	11.4%	Isolates	78	75.7%	014/020	17	21.8%	078/126	12	15.4%
U	34	3.8%	Isolates	33	97.1%	014/020	8	24.2%	078/126	7	21.2%
V	83	9.2%	Isolates	63	75.9%	014/020	10	15.9%	002, 078/126	both n=7	11.1% each
W[3,4]	15	1.7%	Faeces	8	53.3%	011	2	25.0%	several[10]	all n=1	12.5% each
X[3]	19	2.1%	Faeces	18	94.7%	005	4	22.2%	001, 014/020	both n=3	16.7% each
Total	**900**	**100.0%**		**718**	**79.8%**	**014/020**	**140**	**19.5%**	**078/126**	**84**	**11.7%**

[1] Partipation for only 5 months
[2] Missing data on ribotypes of 6 months
[3] Partipation for only 4 months
[4] Missing data on ribotypes of 3 months
[5] 002, 007, 078/126, 150
[6] 011, 013, 115 and unknown
[7] 001, 014/020, 023
[8] 002, 005, 015, 078/126
[9] 005, 050, 078/126
[10] 001, 014/020, 017, 021, 078/126

Results of the ad hoc typing

Healthcare facilities and laboratories using the Reference Laboratory

In the period between May 1st 2018 and May 1st 2019, 12 healthcare facilities and laboratories in the Netherlands sent samples to the Reference Laboratory in Leiden for ad hoc typing (Table 5). The samples were sent for other reasons than for sentinel surveillance, such as severe CDI or suspicion of an outbreak. In total, 138 samples were submitted for ad hoc PCR ribotyping.

Ad hoc ribotyping results

C. difficile could be cultured in 89.1% of the 138 submitted samples. The number of submitted isolates/samples and most common PCR ribotypes stratified per facility/laboratory, are demonstrated in table 5. Ribotype 014/020 was the most commonly found PCR ribotype (15.4%), which was also the most common ribotype in 2017-2018. Other frequently found ribotypes were 002 (8.1%), 015 (8.1%), 001 (6.5%) and 005 (5.7%). The percentage of ribotype 027 was 14.8% (95% CI 7.1-22.6) in 2017-2018 and decreased to 4.1% (95% CI 0.6-7.6) in 2018-2019. The proportion varies in time: 17% in 2016-2017, 20% in 2015-2016, 14% in 2014-2015, 32% in 2013-2014, 20% in 2012-2013, 15% in 2011-2012, 26% in 2010-2011, and 4% in 2009-2010. The percentage of ribotype 078/126 was similar to last year (2018-2019: 4.9% with 95% CI 1.1-8.7, 2017-2018: 8.6% with 95% CI 2.5-14.7). Overall, the most dominant ribotypes of the ad hoc analysis have decreased and the percentage of other ribotypes has increased compared to the previous year. A pie-chart illustrates these findings in comparison to the most common ribotypes of patients included in the sentinel surveillance (Figure 8).

Clinical data

Of the 29 patients with data available, 51.7% had severe CDI in the ad hoc typing analysis. Six of 26 (23.2%) episodes with data available were recurrences. After 30 days, the outcome and course of the disease was known for 27 patients. In total, 3 patients (11.1%) had a complicated course of their CDI infection. These 3 patients all died, of which 2 died partly contributable to CDI (7.4%) and 1 was not related to CDI. No patients were admitted to the ICU as a consequence of CDI and no patients needed surgery as a consequence of CDI.

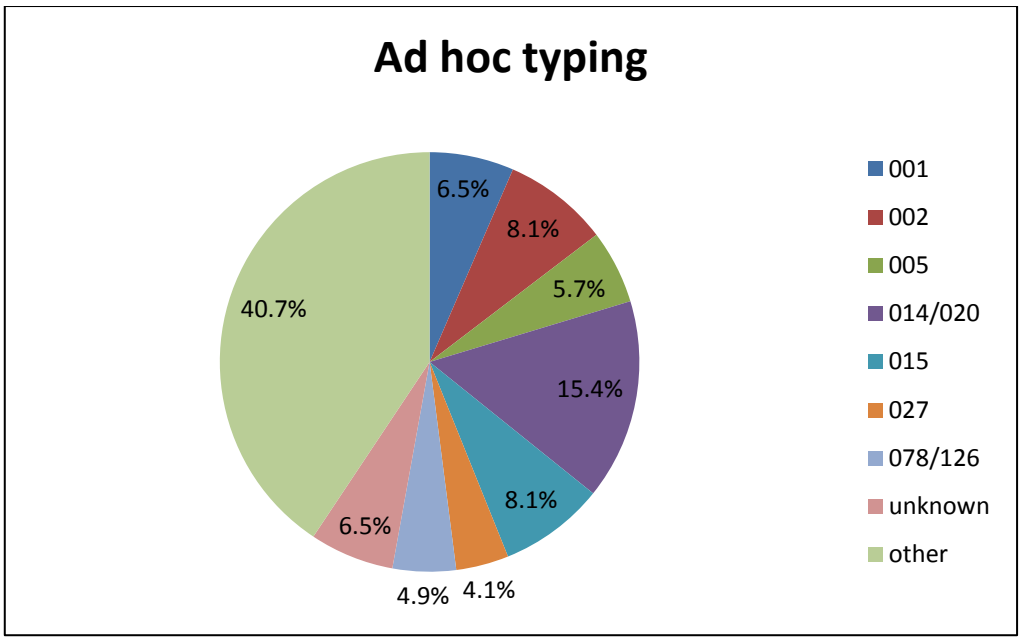
Outbreak investigation

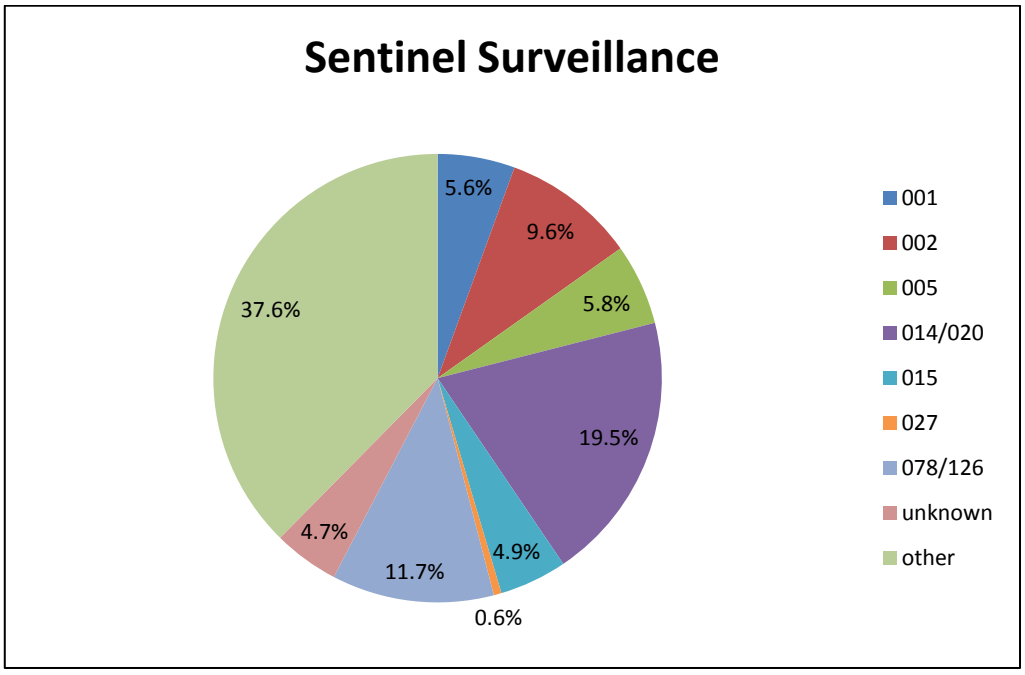
In the period between May 1st 2018 and May 1st 2019, no outbreaks of *C. difficile* in hospitals were reported to the National Reference Laboratory.

Table 5. Results of the ad hoc typing. Period: May 1st 2018 – May 1st 2019. Ribotype 014/020 are indistinguishable by conventional ribotyping, and ribotype 078/126 can be hardly discriminated.

Laboratory / Healthcare facility	Samples		Sample	*C. difficile*		Most common ribotypes		
	N	%	type	N	%		N	%
1	51	37.0%	Faeces	45	88.2%	015	9	20.0%
2	3	2.2%	isolates	2	66.7%	014/020, 131	all=1	all 50.0%
3	6	4.3%	Isolates	6	100.0%	078/126	2	33.3%
4	12	8.7%	Isolates	11	91.7%	027	3	27.3%
5	1	0.7%	Faeces	0	0.0%	-	-	-
6	1	0.7%	Faeces	1	100.0%	001	1	100.0%
7	7	5.1%	Faeces	6	85.7%	002,005,027,042,106,251	all=1	all 16.7%
8	34	24.6%	Isolates	32	94.1%	014/020	6	18.8%
9	1	0.7%	Faeces	1	100.0%	037	1	100.0%
10	18	13.0%	Faeces	16	88.9%	014/020	3	18.8%
11	3	2.2%	Faeces	2	66.7%	023	2	100.0%
12	1	0.7%	Isolates	1	100.0%	194	1	100.0%
Total	**138**			**123**	**89.1%**	**014/020**	**19**	**15.4%**

Figure 8. Proportions of the 5 most frequently encountered PCR ribotypes and ribotype 027 and 078/126 in the sentinel surveillance data, in comparison to ad hoc typing data. Period: May 1st 2018 – May 1st 2019. The category 'other types' consists of 270 different types in the sentinel surveillance data and 50 different PCR-ribotypes in the ad hoc typing data.





Conclusions

The National Reference Laboratory for *C. difficile*

- The Dutch National Reference Laboratory coordinates a sentinel surveillance program with 24 participating acute care hospitals in the Netherlands, and performs molecular characterisation of *C. difficile* in cases of severe CDI or suspected outbreaks ('ad hoc typing service') for other healthcare facilities.
- The Reference Laboratory is now able to recognise 273 different PCR ribotypes.

Results of the sentinel surveillance (May 2018- May 2019)

- Various CDI diagnostic methods are applied, but an increased number (52.1% of participating hospitals) use optimal diagnostic methods as recommended by ESCMID and ECDC. Although recommended, most hospitals do not test all submitted unformed stool samples of hospitalised patients for CDI. This could lead to an underestimation of the incidence, less recognition of CDI in patients who lack traditional risk factors and might also affect the number of complications and mortality.
- A mean incidence rate of 3.17 CDI cases per 10,000 patient-days (varying from 0.95 to 5.88 CDI cases per 10,000 patient-days) was found through sentinel surveillance, similar to last years.
- The disease severity was reported for 877 out of 900 patients included in the surveillance; 16.1% had severe CDI. The 30-day outcome was reported for 812 patients; 90.0% had an uncomplicated course, 0.2% were admitted to the ICU as a consequence of CDI, 0.4% needed surgery as a consequence of CDI and 9.3% of the patients died within 30 days (n=76). For 9 patients (1.1%) their death was known to be contributable to CDI. CDI-contributable mortality was decreased compared to last year. Other outcomes of CDI were comparable to last year. A difference in severity of CDI is observed between the start of the surveillance in 2009-2010 with 27.9%, and 2018-2019 with 16.1%. This may be explained by the decrease in the proportion of ribotype 027.
- The proportion of community-onset cases has increased to 46% of all cases and will be topic for a surveillance in the general population in 2020.
- Similar as in 2017-2018, the most frequent encountered PCR ribotype was ribotype 014/020 (19.5%). Unlike 2017-2018, the second most encountered PCR ribotype was 078/126 (11.7%).
- Ribotype 027 was found in 0.6% of samples (1.2% during May 2017-May 2018).
- No outbreaks were reported this year.

Results of ad hoc typing (May 2018- May 2019)

- Twelve healthcare facilities/laboratories sent 138 samples to the Reference Laboratory for ad hoc typing because of suspected outbreaks, severe CDI cases, or for other reasons.
- Ribotype 014/020 was the predominant ribotype (15.4%), followed by ribotype 002 (8.1%) and ribotype 015 (8.1%).
- Of the patients with data available, 51.7% had severe CDI in the ad hoc typing analysis and 11.1% (3 patients) had a complicated course of their CDI infection due to death. Two patients died partly contributable to CDI (7.4%) and 1 was not related to CDI.
- No outbreaks were reported this year.

Burden of CDI in the Netherlands

- Extrapolating the data of the sentinel surveillance to all hospitals in the Netherlands (with a total of 6,970,901 patient-days per year in the last report[32]), it is estimated that approximately 2,133 hospitalised patients will develop CDI of which 343 will suffer from severe CDI and 23 patients succumb contributable to CDI annually. In these estimations, the impact of CDI in other healthcare facilities than hospitals is not included.

Output of the National Reference Laboratory May 2018-August 2019

Participation of National Reference Laboratory in National and European activities

Granted Tender by ECDC: “Microbiological support to European surveillance of *Clostridium difficile* infections.” 2015-2020.

IMI: Combatting Bacterial Resistance in Europe – *Clostridium difficile* Infections (COMBACTE-CDI). 2017-2020

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Bandelj P, Harmanus C, Blagus R, Cotman M, Kuijper EJ, Ocepek M, Vengust M.Quantification of *Clostridioides (Clostridium) difficile* in feces of calves of different age and determination of predominant *Clostridioides difficile* ribotype 033 relatedness and transmission between family dairy farms using multilocus variable-number tandem-repeat analysis.BMC Vet Res. 2018;14:298.

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Andrés Lasheras S, Martín Burriel I, Aspiroz C, Mainar Jaime RC, Robres P, Sevilla E, Kuijper E, Chirino Trejo M, Bolea R. Incidence and characterization of *Clostridium difficile* in a secondary care hospital in Spain. Rev Esp Enferm Dig. 2019;111:338-344.

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Presentations and posters at congresses

NVMM, voorjaarsvergadering 25-27 April, 2019, Papendal

Poster. Karuna Vendrik, Monique Crobach, Helen Alexandra Shaw, Mark Preston, Brendan Wren, Daan Notermans, Sabine de Greeff, Ed Kuijper. PCR Ribotype 023: Comparable to other hypervirulent ribotypes of *Clostridioides difficile*?

Poster. K.E.W. Vendrik, M.J.T Crobach, C. Harmanus, I.M.J.G. Sanders, D.W. Notermans, S.C. de Greeff, E.M. Terveer, E.J. Kuijper. The epidemiology of *Clostridioides difficile* infections in the Netherlands betweenMay 2017 and May 2018.

Poster. Anoe R Geelen, Quinten R Ducarmon, Bastian VH Hornung, Marie-Astrid Hoogerwerf, Jacqueline J Janse, Elisabeth M Terveer, Romy D Zwittink, Meta Roestenberg, Ed J Kuijper. Isolation and GMP-compliant culturing of a non-toxicogenic *Clostridiodes difficile* strain for application in a controlled human infection model.

29nd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), 13-16 April,2019, Amsterdam.

Oral #O0120;Quinten Ducarmon, Monique Jacqueline Theresia Crobach, Elisabeth Terveer, Céline Harmanus, Ingrid Sanders, Cees Verduin, Ed J. Kuijper, Romy Daniëlle Zwittink.The human gut microbiota during colonisation and infection by *Clostridioides difficile*

Oral #O0133; Elisabeth Terveer,Tom Van Gool, Rogier Ooijevaar, Ingrid Sanders, Bastian Hornung, Josbert Keller, Aldert Bart, Ed J. Kuijper. Human transmission of Blastocystis ST1 and ST3 by faecal microbiota transplantation without development of gastrointestinal symptoms in recipients

P0231. Karuna Vendrik, Monique Jacqueline Theresia Crobach, Alexardra Shaw, Brendan Wren,Ed J. Kuijper.A new hypervirulent PCR ribotype of *Clostridioides difficile* with a unique trehalose genotype in the Netherlands

P0272.Karuna Vendrik, Monique Jacqueline Theresia Crobach, Céline Harmanus, DaanNotermans, Sabine C. De Greeff, Ed J. Kuijper. The epidemiology of *Clostridioides difficile*infections in the Netherlands between May 2017 and May 2018

#P0281 Monique Jacqueline Theresia Crobach, Bastian Hornung,Cees Verduin, Margreet C. Vos, Joost Hopman, Nitin Kumar, Céline Harmanus, Mark Stares, Trevor Lawley, Ed J. Kuijper.Asymptomatic colonisation with *Clostridioides difficile* and onwards transmission in an endemic setting

P0241. Masoumeh Douraghi, Akram Baghani, Amir Aliramezani, Sedighe Ghourchian, Ed J. Kuijper, Malihe Talebi, Farideh Golbabaei, Mahmood Alimohammadi, Mahbobeh Hajabdolbaghi, Alireza Mesdaghinia.Diversity in PCR ribotypes of *Clostridioides difficile* isolates recovered from clinical and non-clinical origins in Tehran, Iran

P0598 Alessio Ciurli, Romy Daniëlle Zwittink, Quinten Ducarmon, Marije Slingerland, Ed J. Kuijper, Martin Giera, Sjaak Neefjes.Metabolomic analysis of the oral cavity during smoking cessation and resumption

P0954. Ilse Michelle Boekhoud, Bastian Hornung, Eloisa Sevilla, Céline Harmanus, Elisabeth Terveer, Rosa Bolea, Jeroen Corver,Ed J. Kuijper,Wiep Klaas Smits. Metronidazole resistance in *Clostridium difficile* is conferred by a plasmid

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Oral: Ed J, Kuijper. From epidemilogyto National Donor Fecesbank.

Oral 11: Ilse Boekhoud, Bastian Hornung, Eloisa Sevilla, Céline Harmanus, Elisabeth Terveer, Rosa Bolea, Jeroen Corver, E.J. Kuijper, Wiep Klaas Smits. Plasmid-mediated metronidazole resistance in C. difficile.

Poster. Michał Piotrowski, Dorota Wultańska, Piotr Obuch-Woszczatyński, Katarzyna Dzierżanowska-FangratWiep Klaas Smits, Ed J. Kuijper, Hanna M. Pituch.Phenotypic and genotypic analysis of Clostridium difficile strains isolated from children hospitalized in a large pediatric center in Poland- retrospectve study.

Poster. Brian A Klein, Colin Lazarra,Hamza Sahil, Mollie Murnane,Gouri Vadali,E.M Terveer,Ed Kuijper, andBruce Roberts. Microbial isolation from freshly-donated versus frozen fecal material of the same donor.

Poster 41. K.E.W. Vendrik. M.J.T. Crobach, Céline Harmanus, I.M.J.G. Sanders, D.W. Notermans, S.C. de Greeff, E.M. Terveer, E.J. Kuijper.Incidence, clinical characteristics and outcome *of Clostridioides difficile* infections in the Netherlands in the period of May 2017-May 2018

Poster 42. K.E.W. Vendrik, M.J.T. Crobach, H.A. Shaw, M.D. Preston, B.W. Wren, D.W. Notermans, S.C. de Greeff, E.J. Kuijper.PCR ribotype 023: a new hypervirulent PCR ribotype of *Clostridioides difficile* in the Netherlands.

Poster 141. Bastian Hornung, E.J. Kuijper, Wiep Klaas Smits.An in silico survey of Clostridioides difficile extrachromosomal elements.

ECDC HAI-Net Clostridioides difficile Infection Network Meeting Stockholm, 22-23 May, 2019

Oral. Ed. J. Kuijper. Options for new chapter(s), e.g. Antimicrobial susceptibility

Oral. Ed. J. Kuijper. Overview of SOPS for CDI surveillance.

Oral. Amoe Baktash. Application of wgMLST for the epidemiology of CDI.

Participations and organization of Workshops and congresses/meetings

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