Twelfth Annual Report of the National Reference Laboratory for *Clostridium difficile* and results of the sentinel surveillance

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Content

Introduction

C. difficile is an anaerobic, spore-forming bacterium which can colonize 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). Alternatives to laboratory diagnosis are endoscopy or histopathology. Cultured isolates can be subtyped by PCR ribotyping. PCR ribotyping uses the type-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 recognize 263 different PCR ribotypes.

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 48hrs after the last diarrhoeal symptoms, although it is known that CDI patients may shed spores for a prolonged amount of time.[8] Possibly, recommendations on the handling of asymptomatic *C. difficile* carriers will change in the coming years as more evidence on the efficacy of isolation measures for these patients accumulates.

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.[9] 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.[10] Similarly, a critical review from a study group in the Netherlands also concluded that vancomycine is preferred as first agent of choice.[11]

Despite antibiotic therapy, CDI recurrence is common. Recently, human monoclonal antibodies against *C. difficile* toxin B have been tested in a clinical setting to prevent recurrent CDI.[12] Fecal 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, fecal microbiota transplantation is often not offered despite an indication for it. To overcome these problems, the National Donor Feces 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 standardized protocol including microbiological investigations of serum and feces. Stool preparations of these healthy donors are stored at the LUMC. These ready-to-use frozen donor feces suspensions can be ordered by treating physicians of patients with recurrent or severe CDI (info@ndfb.nl). Patients can receive the microbiota transplantation at their local hospital. A total of n=79 fecal microbiota

transplantations for recurrent or severe CDI with a feces suspension from the NDFB were performed in the period May 2016-May 2018 with a cure rate of 89%.

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[15] and rapidly spread within Netherlands while causing major outbreaks.[16,17] Retrospectively, the rapid spread of the ribotype 027 strain across Northern-America and Europe has been attributed to its high level of fluoroquinolone resistance.[18] A recent study suggests that the rapid spread might also be attributed to a different trehalose metabolism in ribotype 027 strains, which causes the ability to metabolize 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.[19] 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[16,20], which may reflect type-specific host susceptibility and/or an increased virulence of the strain.[21] Since mid-2006, the occurrence of ribotype 027 in the Netherlands has decreased significantly.[22] The CDI incidence rate has stabilised at 3 CDI cases per 10.000 patient-days.[23]

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 Center 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*. Currently, 22 acute care hospitals are participating in the sentinel surveillance programme voluntary. Each year, results are reported on the website of the National Institute for Public Health and the Environment (RIVM).[23] This current report is the twelfth 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 2017 and May 1st 2018.

The Netherlands is also participating in the European-wide CDI surveillance which is led by ECDC. The protocol for this European surveillance program is available at http://ecdc.europa.eu/en/publications/Publications/Clostridium-difficile-infections-surveillance-protocol-version-2.3.pdf.

Aims and procedures of the sentinel surveillance

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

1. To obtain continuous incidence rates of patients with CDI in participating hospitals in the Netherlands.
2. To identify and characterize 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 hospitalized patients >2 years with clinical sign 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 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=13) send strains to the laboratory of the Leiden University Medical Center. Other laboratories (n=7) send faecal samples. Some laboratories (n=2) send faeces 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.[24,25] In the OSIRIS system, the results of the PCR ribotyping 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.

Microbiological reports

All faecal samples are cultured and *C. difficile* isolates are characterized (see next chapter) at the laboratory of the Leiden University Medical Center. In case PCR ribotype 027 is found, the local microbiologist is directly informed by telephone and asked if there is a need for additional information or advice. Once a week, microbiological results are sent by e-mail to the submitting microbiologist, infection control practitioners, and to CIb when an outbreak is suspected or ribotype 027 isolated. The results are also reported in OSIRIS. All submitting laboratories 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.

Incidence rates and outbreaks

The last data-extraction for this annual report was performed on July 12th 2018. 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 for Jan-Apr 2018, the data from Jan-Apr 2017 were used as denominator. If no data were supplied by the hospital, data were acquired from jaarverslagenzorg.nl.[26] 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 nursing homes.
2. To isolate *C. difficile* for further typing from faeces samples of patients with CDI sent to the reference laboratory by laboratories that do not culture *C. difficile*.
3. To characterize isolated *C. difficile* strains by PCR ribotyping, and if required toxinotyping, presence of genes *tcd*A and *tcd*B, presence of binary toxin genes and the presence of deletions in *tcd*C.
4. To report the results of the investigation to CIb and to medical microbiologists who submitted the samples from severe CDI diseases or outbreaks.
5. To obtain demographical data and clinical information of the patients with microbiological proven CDI.

C. difficile isolation

Isolation of *C. difficile* from faeces 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*.[25] 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.[27] Deletions in *tcdC* can be determined by PCR using in-house designed primers.

C. difficile Reference Library

The Reference Laboratory added 14 new ribotypes to the Reference Library in the prior year, and is now able to recognize 263 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 is found, the laboratories are also informed by telephone and are offered to contact the LUMC or CIb for additional information and advices. Submitting laboratories also receive an official report by regular post.

Collection of patient data

A standardized 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.[24,25] 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 current 22 participating hospitals of the sentinel surveillance programme. Both university hospitals (n=5) and primary or secondary care hospitals (n=17) 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 by May 2018. 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 2018, 9/22 hospitals (41%) used an ESCMID recommended algorithm, which is less than last year (54%). Another 12 hospitals (55%) used 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. Five of the 12 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. By May 2018, 5 of the 22 hospitals (23%) tested all submitted unformed stool samples from hospitalized patients 2 years or older for CDI. Nine out of 22 hospitals (41%) tested unformed stool samples from patients admitted for at least 3 days (the so-called 3-day rule) or with a specific request for CDI testing. Another 7 hospitals (32%) 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 7.0% (range 2.1-11.3%; Table 3).

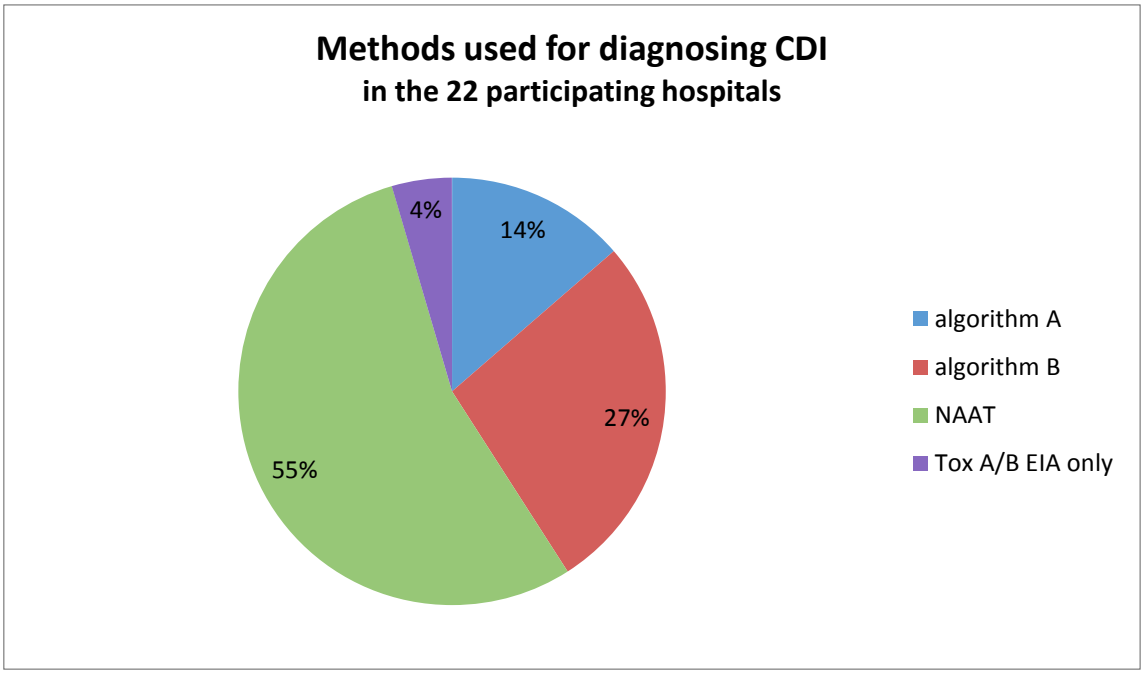


Figure 2. Laboratory methods used for diagnosing CDI in the 22 hospitals participating in the sentinel surveillance program. Algorithm A and B are recommended methods, all the others are non-recommended methods.

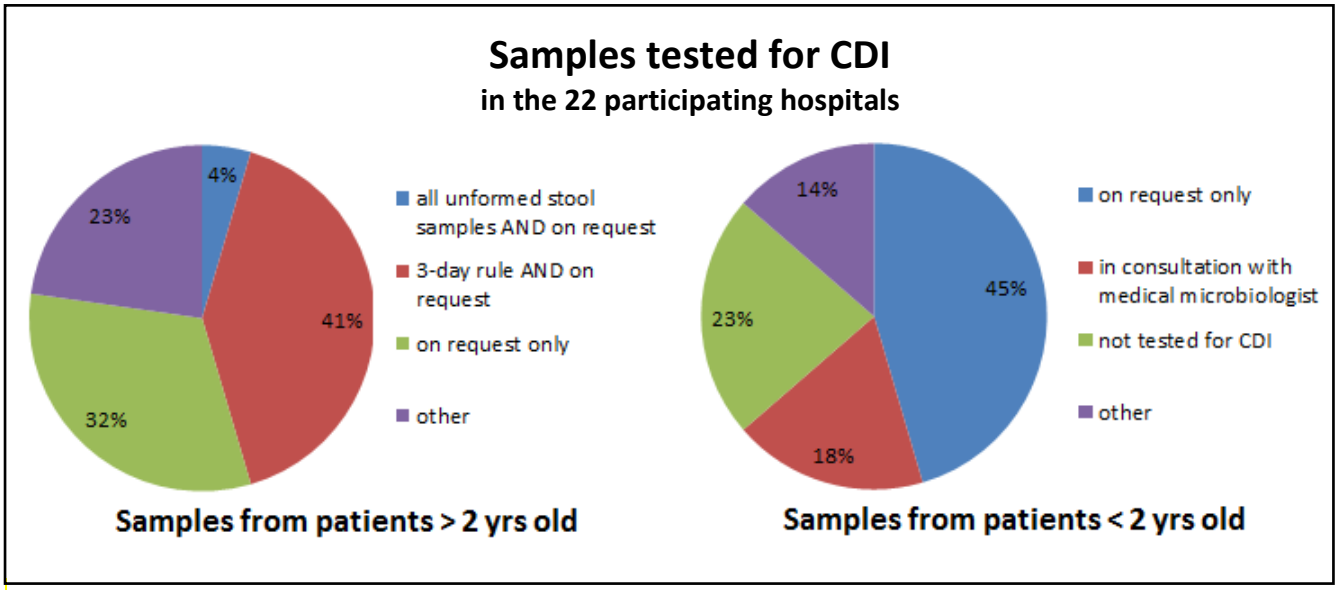


Figure 3. Samples tested for CDI in the 22 hospitals participating in the sentinel surveillance. Selection criteria for samples from patients >2 years are shown on the left, selection criteria for samples from patients <2 years are shown on the right.

Incidence in participating hospitals

The numbers of CDI per 10.000 patient-days per hospital are shown in Table 3, and compared to the incidence rate of the preceding year. The mean incidence was 2.90 CDI cases per 10.000 patient-days (varying from 0.65 to 5.08 CDI cases per 10.000 patient-days), comparable to the incidence of 3.03 that was reported in 2016-2017.[23] For hospitals that submitted data on monthly patient-days (19 hospitals), the overall monthly CDI incidence rates were calculated over the year (see Figure 4).

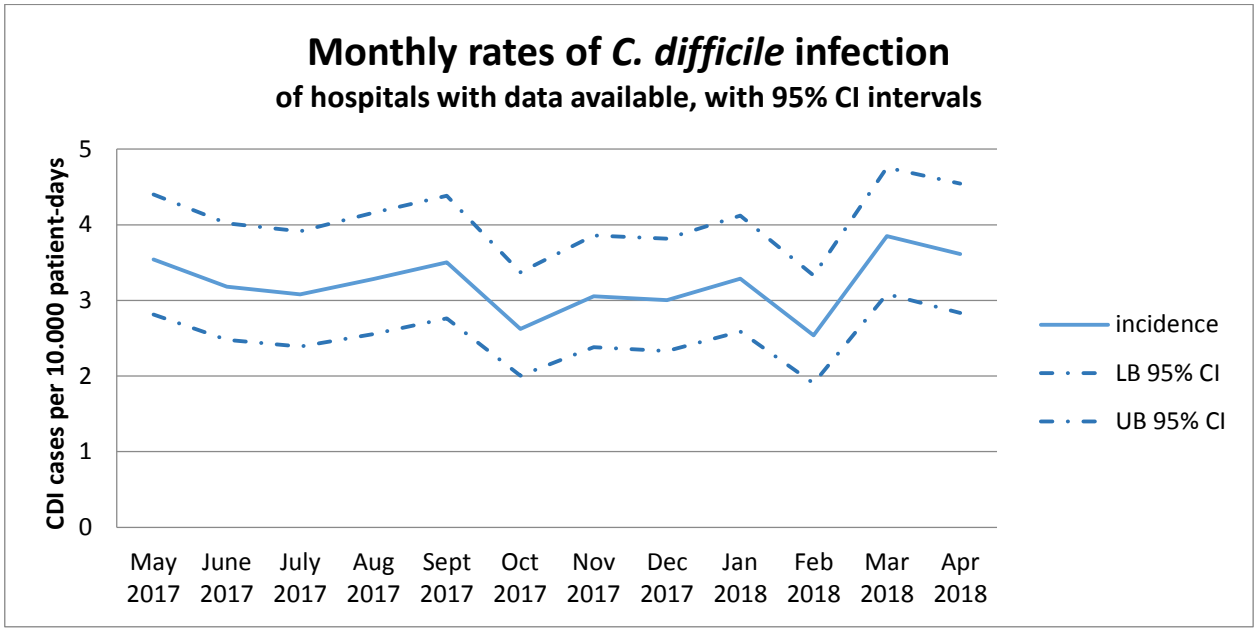


Figure 4. Monthly rates of *C. difficile* infection (cases per 10,000 patient-days) in 19 of the participating hospitals. LB 95% CI; lower bound 95% confidence interval, UB 95% CI; upper bound 95% confidence interval.

Submitted strains for PCR ribotyping

Of 879 CDI patients included in sentinel surveillance between May 1st 2017 and May 1st 2018, 683 *C. difficile* isolates could be PCR ribotyped and linked to the clinical data (78%). 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 or not sending the isolates or faeces to the National Reference Laboratory (n=122) or the inability to type *C. difficile* at the National Reference laboratory (culture negative or other *Clostridium* species; n=74).

Circulating PCR ribotypes

Similar as the previous year, ribotype 014/020 was the most frequently isolated ribotype.
This year ribotype 002 was the second most frequently isolated ribotype, in contrast to last year when this was ribotype 078/126.
Ribotype 014/020 (indistinguishable by conventional PCR ribotyping) was isolated in 143 of the 683 samples (20.9%, 95% CI 17.9-24.0). Ribotype 002 was found in 81 isolates (11.9%; 95% CI 9.4-14.3). The closely related ribotypes 078 and 126 were found in 68 samples (10.0%; 95% CI 7.7-12.2), ribotype 001 in 56 isolates (8.2%; 95% CI 6.1-10.3), and ribotype 005 in 31 isolates (4.5%; 95% CI 3.0-6.1). Eight isolates were identified as ribotype 027 (1.2%; 95% CI 0.4-2.0). Of 25 isolates (3.7%, 95% CI 2.3-5.1) the PCR ribotype pattern was not recognized in our database. Of these isolates, 2 pairs of unknown ribotypes were exactly the same. The results stratified per participating centre are displayed in Table 4. A pie-chart of the five most common ribotypes and ribotype 027 of patients included in the sentinel surveillance is illustrated in Figure 7.

Changes in circulating PCR ribotypes

In Figure 5, the proportions of the 5 most common ribotypes are shown in time.
The proportion of ribotype 002 was significantly increased compared to the previous years (2017-2018 95% CI 9.4-14.3, 2016-2017 95% CI 4.8-8.1). Ribotype 014/020 had a proportion of 11.9% at the start of the surveillance in 2009-2010 (95% CI 9.1-14.7) and a proportion of 20.9% in 2017-2018 (95% CI 17.9-24.0). In the previous year there was an outbreak of ribotype 001 with an increased proportion of ribotype 001. This year, the proportion of ribotype 001 was slightly decreased compared to last year, but remained significantly higher compared to 2015-2016 (2017-2018 95% CI 6.1-10.3, 2016-2017 95% CI 8.2-12.2, 2015-2016 95% CI 2.1-4.7).
The proportion of ribotype 078/126 was not significantly different from the previous year (2017-2018 95% CI 7.7-12.2, 2016-2017 95% CI 10.0-14.3).
The proportion of ribotype 027 was also not significantly different from last year (2017-2018 95% CI 0.4-2.0, 2016-2017 95% CI 0.1-1.1). 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 6 individual cases in 3 hospitals (3/22; 13.6%). Two patients had 2 CDI episodes with ribotype 027.

(Suspected) outbreaks in participating hospitals

In the period between May 1st 2017 and May 1st 2018, no outbreaks of *Clostridium difficile* in hospitals participating in the sentinel surveillance were reported to the National Reference Laboratory.

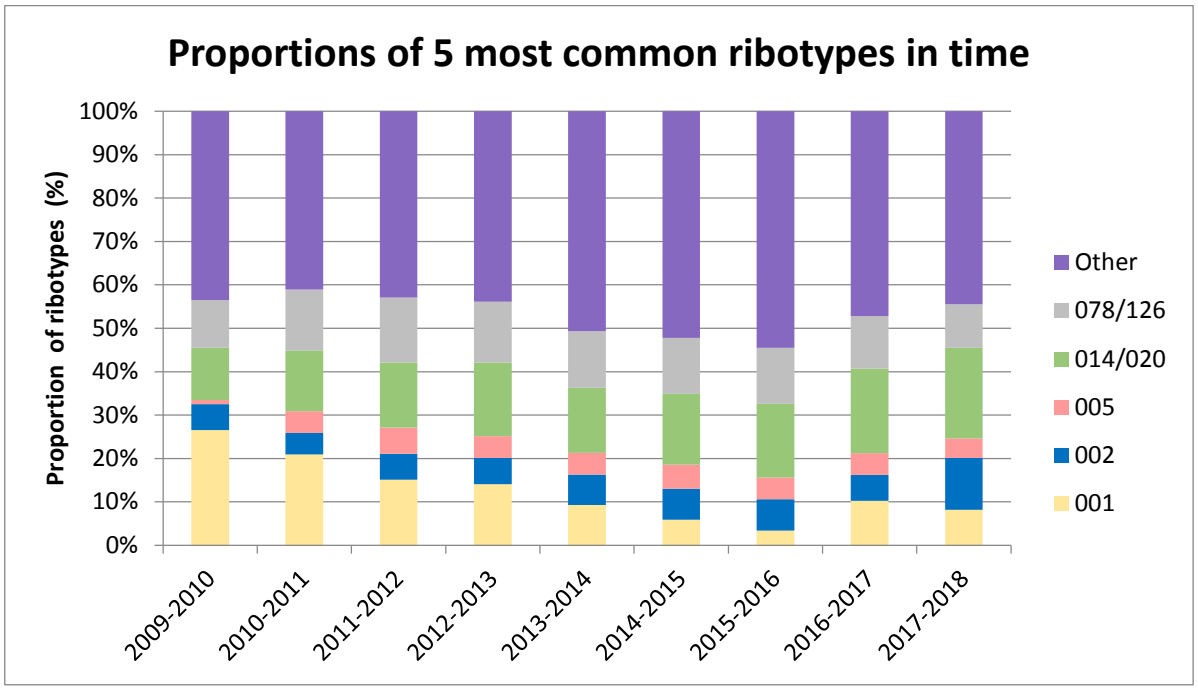


Figure 5. Proportion of the 5 most common ribotypes in time in sentinel surveillance samples.

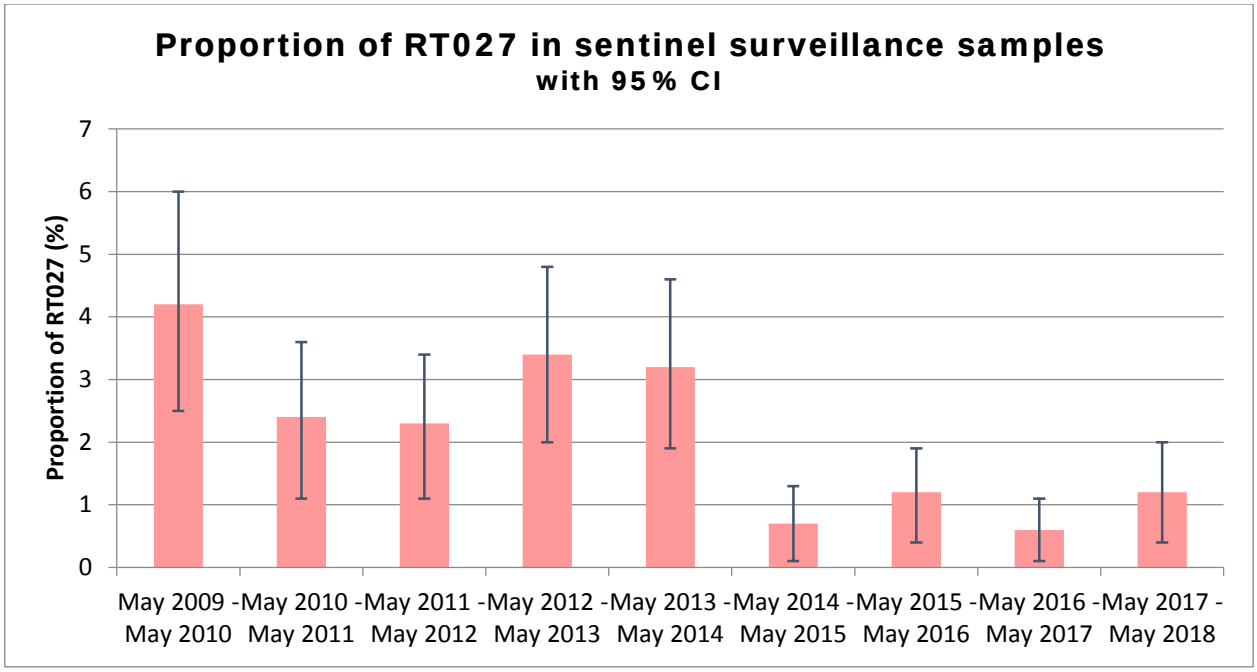


Figure 6. Proportion of 027 in sentinel surveillance samples. CI; confidence interval.

Demographical and clinical data

Demographical and clinical characteristics were collected from 879 patients included in the sentinel surveillance (Table 1). The mean age was 66.0 years (95% CI 64.8-67.3). Of all patients, 3.0% (n=26) was younger than eighteen years old and 63.0% (n=551) was older than 65 years old. Furthermore, 45% of the patients had a community-onset of symptoms and 55% a healthcare facility-onset of symptoms. A total of 175 patients (20.4%) had severe CDI, defined as bloody diarrhoea and/or diarrhoea with hypovolemia or hypoalbuminemia (<20g/L) and/or with fever (T >38.0 $^{\circ}$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 797 patients. In total 692 patients (86.8%) had an uncomplicated course of their CDI infection. On the other hand, 2 patients (0.3%) were admitted to the ICU as a consequence of CDI, 3 patients (0.4%) needed surgery as a consequence of CDI and 100 patients with CDI (12.5%) died. Twenty five deaths (3.1%) 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. Also, the proportion of patients with severe CDI and proportion of patients with a complicated course of CDI were comparable. Furthermore, CDI-related and overall mortality in CDI patients were not significantly different from the previous year. The proportion of community-onset cases was 37% at the start of the surveillance in 2009-2010 (95% CI 32.9-41.1) and 45% in 2017-2018 (95% CDI 41.7-48.3).

Table 1. Clinical characteristics and outcome of patients participating in the sentinel surveillance (n=879)

Patient characteristics and outcome	n/n[a]	%
Gender female	438/875	50,1%
Location of onset CDI		
hospital	436/873	49,9%
at home	393/873	45,0%
nursing home	22/873	2,5%
other health-care facility	22/873	2,5%
Hospital department		
Internal Medicine	164/436	37,6%
Surgery	69/436	15,8%
Lung diseases and TB	32/436	7,3%
Geriatrics	16/436	3,7%
Gastroenterology	36/436	8,3%
Cardiology	37/436	8,5%
ICU	18/436	4,1%
Neurology	20/436	4,6%
Pediatrics	9/436	2,1%
Other or unknown	35/436	8,0%
Antibiotics prior to CDI	526/815	64,5%
Recurrence	166/653	25,4%
Severe CDI	175/857	20,4%
Pseudomembranous colitis	23/857	2,7%
Hypovolemia or hypo-albuminaemia	98/857	11,4%
Bloody diarrhoea	38/857	4,4%
Fever and leucocytosis	67/857	7,8%
Outcome		
Uncomplicated	692/797	86,8%
Surgery needed	3/797	0,4%
ICU admission needed	2/797	0,3%
Death, contributable to CDI	25/797	3,1%
Death, unrelated to CDI	67/797	8,4%
Death, cause unknown	8/797	1,0%

Table 2. Data from the sentinel surveillance for the period May 2017-May 2018 compared to the data from preceding years. The bottom line shows the number of outbreaks that were identified by ad hoc typing.

Surveillance period (May-May)	2009-2010	2010-2011	2011-2012	2012-2013	2013-2014	2014-2015	2015-2016	2016-2017	2017-2018
Incidence									
per 10.000 patient-days	2,7	2,8	2,9	2,9	2,9	3,0	3,1	3,0	2,9
Location of onset									
within healthcare facility	63%	73%	69%	63%	64%	59%	58%	59%	55%
at home	37%	27%	31%	37%	36%	41%	42%	41%	45%
Course and outcome									
Severe CDI	28%	20%	27%	25%	21%	24%	21%	17%	20%
Uncomplicated course	66%	86%	87%	88%	87%	86%	89%	87%	87%
Deaths contributable to CDI	4%	3%	4%	2%	3%	4%	2%	2%	3%
PCR ribotype 027									
Prevalence	4.2%	2.4%	2.3%	3.4%	3.2%	0.7%	1.2%	0.6%	1.2%
N reported 027 outbreaks-sentinel surveillance	1	1	0	1	0	0	0	0	0
N reported 027 outbreaks-ad hoc typing	2	2	1	2	5	1	0	1	1

Table 3. Number of patients included in the sentinel surveillance per hospital, and incidence data. Period: May 1st 2017 – May 1st 2018. 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 2017-2018	Incidence per 10.000 PD 2016-2017	Incidence difference
A	algorithm B	on request only	4.4% (38/865)	15284	0,65	1,00	-0,35
B	algorithm B	all unformed stool samples AND on request	2.1% (10/478)	3561	1,17	0,73	0,44
C	algorithm B	3-day rule AND on request	6.1% (19/312)	5372	1,40	2,11	-0,71
D	toxin A/B EIA	on request only	3.7% (71/1933)	7671	1,85	2,88	-1,03
E	algorithm A	3-day rule AND on request	2.6% (8/304)*	6230	1,87	0,68	1,19
F	algorithm B	other criteria[1]	4.2% (29/689)	5488	2,13	1,28	0,85
G	NAAT**	3-day rule AND on request	7.6% (42/553)	9121	2,19	1,75	0,44
H	algorithm A	3-day rule AND on request	9.1% (98/1081)	15024	2,27	3,38	-1,11
I	algorithm A	3-day rule AND on request	2.5% (60/2440)	10570	2,29	1,87	0,41
J	algorithm C/per 15-3-18 NAAT	other criteria[2]	7.6% (161/2112)	11418	2,41	2,52	-0,11
K	NAAT**	on request only	11.3% (55/488)	5589	2,98	3,71	-0,73
L	NAAT	on request only	7.6% (163/2150)	14449	3,06	1,99	1,06
M	NAAT	on request only	9.0% (135/1495)	12019	3,33	3,99	-0,66
N	NAAT**	on request only	6.3% (237/3786)	10147	3,45	2,75	0,70
O	NAAT	3-day rule AND on request	8.2% (91/1114)	12782	3,52	4,51	-0,99
P	algorithm B	3-day rule AND on request	10.5% (156/1484)	12597	3,64	4,25	-0,61
Q	NAAT	3-day rule AND on request	8.3% (121/1462)	13473	3,83	3,40	0,43
R	NAAT	3-day rule AND on request	10.6% (206/1936)	15032	3,88	6,62	-2,74
S	algorithm B	on request AND if unformed only	9.8% (72/738)	8281	3,92	4,90	-0,97
T	NAAT**	on request only	7.9% (154/1948)	17317	3,99	4,43	-0,44
U	NAAT	other criteria[6]	NA	6766	4,93	4,56	0,36
V	NAAT**[3]	all unformed stool samples from inpatients[4]	7.0% (328/4670)	18860	5,08	2,78	2,30
Total			**7.0%**		**2,90**	**3,03**	**-0,13**

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 from 01-01-2017 untill 01-01-2018, **and culture of positive samples

[1] all unformed stool samples from inpatients and samples from immunocompromised patients, from patients with acute diarrhea, during increased CDI incidence or on request

[2] all unformed stool samples from inpatients, samples from outpatients if CDI test is requested

[3] during weekend days screening with GDH and Tox A/B EIA

[4] stool samples from GPs only tested on request or if patients have used antibiotics

[5] or in consultation with medical microbiologist

[6] all unformed stool samples from inpatients, on request or if clinical information states that patients have used antibiotics

[7] unless immunocompromised and if requested only

Table 4. The two most frequently found ribotypes per hospital, isolated amongst patients that were included in the sentinel surveillance. Period: 1st 2017 – May 1st 2018. 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	12	1,4%	Isolates	11	92%	014/020	3	27%	015	2	18%
B	5	0,6%	Isolates or faeces	5	100%	several[2]	all n=1	20% each	-		0%
C	9	1,0%	Faeces	7	78%	several[3]	all n=1	14% each	-		0%
D	17	1,9%	Faeces	10	59%	002	2	20%	several[4]	all n=1	10% each
E	14	1,6%	Isolates	4	29%	several[1]	all n=1	25% each	-		0%
F	14	1,6%	Isolates or faeces	13	93%	002	4	31%	078/126	3	23%
G	24	2,7%	Isolates	14	58%	002	4	29%	014/020	3	21%
H	41	4,7%	Isolates	31	76%	014/020	11	35%	002	5	16%
I	29	3,3%	Isolates	26	90%	014/020	6	23%	078/126	5	19%
J	33	3,8%	Faeces	31	94%	002	7	23%	014/020	6	19%
K	20	2,3%	Isolates	11	55%	002	4	36%	078/126	2	18%
L	53	6,0%	Faeces	42	79%	014/020	10	24%	078/126	6	14%
M	48	5,5%	Isolates	29	60%	014/020	5	17%	002	4	14%
N	42	4,8%	Isolates	41	98%	014/020	11	27%	002	7	17%
O	54	6,1%	Isolates	41	76%	014/020	7	17%	001	5	12%
P	55	6,3%	Faeces	45	82%	several[5]	all n=8	18% each	023	4	9%
Q	62	7,1%	Isolates	41	66%	014/020	11	27%	027	5	12%
R	70	8,0%	Faeces	58	83%	001	15	26%	014/020	13	22%
S	39	4,4%	Faeces	36	92%	014/020	8	22%	002	4	11%
T	83	9,4%	Isolates	60	72%	014/020 and 078/126	both n=11	18% each	001 and 002	both n=6	10% each
U	40	4,6%	Isolates	37	93%	014/020	9	24%	078/126	5	14%
V	115	13,1%	Isolates	90	78%	014/020	16	18%	002	9	10%
Total	**879**	**100%**		**683**	**78%**	**014/020**	**143**	**20,9%**	**002**	**81**	**11,9%**

*Dominant sample type send to LUMC

[1] 002, 005, 050, 163

[2] 014/020, 017, 053, 062, 079

[3] 005, 012, 14/020, 050, 220, 351 and unknown

[4] 001, 005, 014/020, 018, 021, 023, 024 and 078/126

[5] 002, 014/020, 078/126

Results of the ad hoc typing

Healthcare facilities and laboratories using the Reference Laboratory

In the period between May 1st 2017 and May 1st 2018, 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, 92 samples were submitted for ad hoc PCR ribotyping.

Ad hoc ribotyping results

C. difficile could be cultured from 88% of the 92 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 (26%), while in the previous year this was ribotype 027. Other frequently found ribotypes were 027 (15%), 002 (11%) and 078/126 (9%). The percentage of ribotype 027 was not significantly different compared to last year. 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 also not significantly different from last year. A pie-chart illustrates the differences of these findings in comparison to the five most common ribotypes of patients included in the sentinel surveillance (Figure 7).

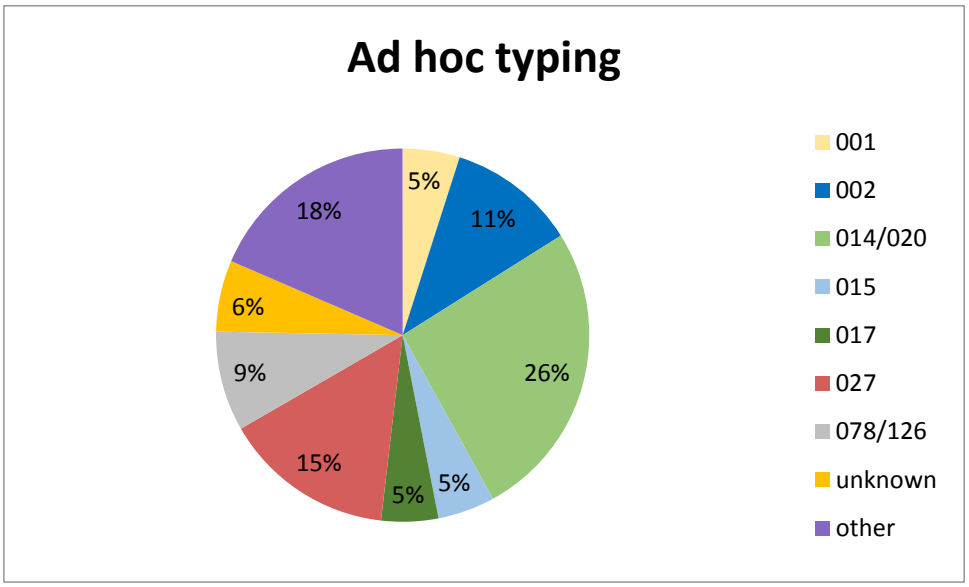
Outbreak investigation

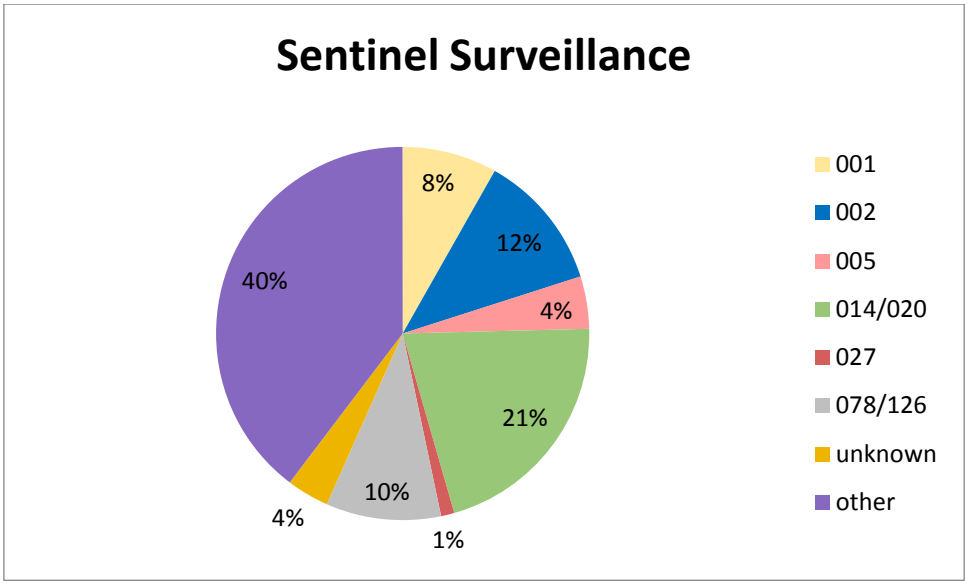
During June/July 2017, one outbreak due to ribotype 027 was reported in a hospital in the southwestern part of the Netherlands. Within 2 months, 6 patients were reported, and another 2 cases linked to the outbreak were reported in the following months.
Another outbreak (involving 15 patients) due to ribotype 017 took place in one hospital in the northwestern part of the Netherlands.

Table 5. Results of the ad hoc typing. Period: May 1st 2017 – May 1st 2018. 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	62	67%	feces	55	89%	014	14	25%
2	1	1%	isolates	1	100%	057	1	100%
3	5	5%	isolates	5	100%	014	2	40%
4	9	10%	isolates/feces	8	89%	014,027	all n=2	all 25%
5	1	1%	feces	1	100%	024	1	100%
6	1	1%	feces	1	100%	014	1	100%
7	2	2%	feces	0	0%	-	-	-
8	1	1%	feces	0	0%	-	-	-
9	1	1%	feces	1	100%	018	1	100%
10	3	3%	isolates/feces	3	100%	002,017,170	all n=1	all 33%
11	4	4%	isolates	4	100%	017	3	75%
12	2	2%	isolates	2	100%	002,014	all n=1	all 50%
Total	**92**			**81**	**88%**	**014/020**	**21**	**26%**

Figure 7. Proportions of the five most frequent encountered PCR ribotypes and ribotype 027 for sentinel surveillance data, in comparison to ad hoc typing data. Period: May 1st 2017 – May 1st 2018. The category 'other types' consists of 271 different types in the sentinel surveillance data and 15 different PCR-ribotypes in the ad hoc typing data.





Conclusions

The National Reference Laboratory for *C. difficile*

- The National Reference Laboratory coordinates a sentinel surveillance program with 22 participating acute care hospitals in the Netherlands, and performs molecular characterisation of *C. difficile* in cases of severe *C. difficile* infections (CDI) or suspected outbreaks ('ad hoc typing service') for other healthcare facilities.
- The Reference Laboratory is now able to recognize 263 different PCR ribotypes.

Results of the sentinel surveillance (May 2017- May 2018)

- Diverse CDI diagnostic methods are applied, and less than half of hospitals participating in the sentinel surveillance use optimal diagnostic methods as recommended by ESCMID and ECDC. In most cases, this could lead to an overestimation of the incidence, due to the detection of *c. difficile* carriers. Although recommended, most hospitals do not test all submitted unformed stool samples of hospitalized 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 2.90 CDI cases per 10.000 patient-days was found through sentinel surveillance (varying between hospitals from to 0.65 to 5.08 CDI cases per 10.000 patient-days), similar to last years.
- The disease severity was reported for 857 out of 879 patients included in the surveillance; 20% had severe CDI. The 30-day outcome was reported for 797 patients; 87% had un uncomplicated course, 0.3% was admitted to the ICU due to CDI, 0.4% needed surgery because of CDI and 12.5% of the patients died within 30 days (n=100). For 25 patients (3.1%) their death was known to be contributable to CDI. Outcomes of CDI were comparable to last year.
- The proportion of community-onset cases has increased compared to the start of the surveillance.
- Similar as in 2016-2017, the most frequent encountered PCR ribotype was ribotype 014/020 (20.9%). Unlike 2016-2017, the second most encountered PCR ribotype was 002 (11.9%).
- Ribotype 027 was found in 1.2% of samples (0.6% during May 2016-May 2017).

Results of ad hoc typing (May 2017- May 2018)

- Twelve healthcare facilities/laboratories sent 92 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 (26%), followed by ribotype 027 (15%) and ribotype 002 (11%).
- Two outbreaks were reported this year, one with ribotype 027 and one with ribotype 017.

Burden of CDI in the Netherlands

- Extrapolating the data of sentinel surveillance to all hospitals in the Netherlands (with a total of 9.400.000 patient-days per year[28]), it is estimated that approximately 2707 hospitalized patients will develop CDI, and 84 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 2017-July 2018

Publications related to the reference laboratory

Kenters N, Huijskens EGW, de Wit SCJ, Sanders IGJM, van Rosmalen. Effectiveness of various cleaning and disinfectant products on *Clostridium difficile* spores of PCR ribotypes 010, 014 and 027. Antimicrob Resist Infect Control. 2017 Jun 3;6:54. doi: 10.1186/s13756-017-0210-3.

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Participation of National Reference Laboratory in National and European activities

Granted Tender by ECDC: "Microbiological support to European surveillance of *Clostridium difficile* infections." 2015-2019.

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

Presentations and posters at congresses

NVMM, Annual meeting, Papendal, 27-28 March 2018

O019. I. Boekhoud, E. van Eij1, E. Kuijper, I. Sanders, G. Wright, W.K. Smits Transcriptional response of *Clostridium difficile* to sub-inhibitory concentrations of antimicrobial compounds

O052 E.M. Terveer, E. Boeije-Koppenol, B. Goorhuis, R. Ooijevaar, M.P. Bauer, E. Nood van, Y.H. Beurden van, C.M.J.E. Vandenbroucke-Grauls, H.W. Verspaget, J.J. Keller, E.J. Kuijper. Two years of experiences of the Netherlands Donor Feces Bank

O057. M. Crobach. Standardised diagnostics of CDI in The Netherlands and Europe.

P016. I.M.J.G. Sanders, E.M. Terveer, E.J. Kuijper, E.C.J. Claas. Molecular point-of-care diagnostics for *Clostridium difficile* in 20 minutes.

P023. B.V.H. Hornung, E.J. Kuijper, W.K. Smits. An in silico survey of *Clostridium difficile* plasmid epidemiology.

28th European Congress of Clinical Microbiology and Infectious Diseases. Madrid, 21-24 April 2018

O0330. M. Crobach, E. Terveer, J. Hopman, M. Vos, E. Kuijper. Asymptomatic *Clostridium difficile* colonization and risk of CDI: a multicentre study

O0257. M. Crobach, C. Harmanus, I. Sanders, E. Terveer, S. de Greeff, D. Notermans, S. Van Dorp, E. Kuijper. Epidemiology of *Clostridium difficile* infections in the Netherlands May 2016-May 2017

P0760. B. Hornung, J. Norman, E. Terveer, B. Roberts, J. Keller, E. Kuijper. Developing a defined drug from faecal microbiota transplant: an ongoing challenge.

P0383. E. Novakova, M. Krutova, O. Nyc, E. Kuijper, M. Garabasova, M. Novak, N. Kotlebova, M. Stefkovicova. A high prevalence of Clostridium difficile ribotypes 001 and 176 recognized within an enhanced option of European standardized *Clostridium difficile* infection surveillance in Slovakia, 2016.

P0397. M. Kachrimanidou, O. Tsachouridou, I. Ziogas, E. Christaki, E. Protonotariou, M. Symeon, L. TOPTSI, L. Skoura, E. Kuijper. *Clostridium difficile* infections in a university hospital in Greece are mainly associated with PCR ribotypes 017 and 126.

P0384. M. Crobach, C. Harmanus, I. Sanders, E. Terveer, S. de Greeff, D. Notermans, S. Van Dorp, E. Kuijper. Community-onset versus hospital-onset *Clostridium difficile* infection: does it matter?

P0392. A. Budimir, I. Mareković, M. Mijač, Z. Bosnjak, M. Payerl-Pal, E. Susic, I. Matas, A. Novak, C. Harmanus, E. Kuijper. Epidemiology of *Clostridium difficile* infections in Croatia-national study.

P0382. M. Krutova, J. Matejkova, F. Prusik, E. Nycova, V. Paleckova, V. Vanis, D. Nemcova, M. Curdova, P. Jezek, L. Geigerova, E. Zalabska, M. Bohackova, A. Kucharova, D. Vesela, A. Melichar, D. Zamazalova0, E. Miskova, E. Kuijper, O. Nyc. A reduced susceptibility to metronidazole and vancomycin and high resistance to moxifloxacin were revealed within an enhanced option of European standardized *Clostridium difficile* infection surveillance in the Czech Republic, 2016.

Invited presentations

3rd *Clostridium difficile* day. 13 May 2017-Prague, Czech Republic; Ed J. Kuijper et al. Update of CDI in Europe.

Stockholm, Karolinska Institute 4 May 2917; Ed J. Kuijper et al. *Clostridium difficile* infections. New Laboratory and Clinical Data.

International Conference on Infection Prevention and Control, 20 June to 23 June 2017 Geneva. N. Kenters, E. Huijskens, S. De Wit, I. Sanders, J. Van Rosmalen, E. Kuijper, A. Voss; Effectiveness of various cleaning and disinfectans products on *Clostridium difficile* spores of PCR ribotype 010. 014 and 027.

Basel, Joint annual meeting 2017 of the Swiss Societies for Microbiology (SSM), Infectious Diseases (SSI), Hospital Hygiene (SSHH), Tropical Medicine and Parasitology (SSTMP) and the Swiss Society of Tropical and Travel Medicine (SSTTM). August 30 - September 01, 2017. Ed J. Kuijper et al. CDI diagnosis and molecular typing: What's new?

25th United European Gastroenterology, 28 October-1 November 2017,Barcelona. J.J Keller and Ed J. Kuijper; Update of CDI treatment,

Madrid, 17th November 2017. MSD Global Infectious Disease Forum. Ed J. Kuijper et al. What is Recurrent *C difficile* Infection and How Common is it?

Marseille, 13 December 2017. Ed J. Kuijper et al. About *Clostridioides difficile* infections and development of "poop banks".

Participation and Organization of Workshops

Vienna ECDC, 11-12 May 2017. Train-the-trainer workshop for Microbiological support to European surveillance of *Clostridium difficile* infections.

Annual meeting of the Reference Laboratory with all participating Laboratories, 23 November 2017, LUMC.

18-19 January 2018. Landelijke Cursus Infectiepreventie, Oirschot. Ed J. Kuijper et al. *Clostridium difficile* infecties.

February 1th, 2018. Regional Infection Prevention Control meeting in Leiden. Ed J. Kuijper et al. New aspects on CDI Prevention and Control.
7TH Next Generation Sequence Workshop on *Neisseria gonorrhoeae* and *Clostridium difficile*. Vienna, Austria 21- 23. March 2018. Ed J. Kuijper et al. Application of NGS for *C. difficile.*

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