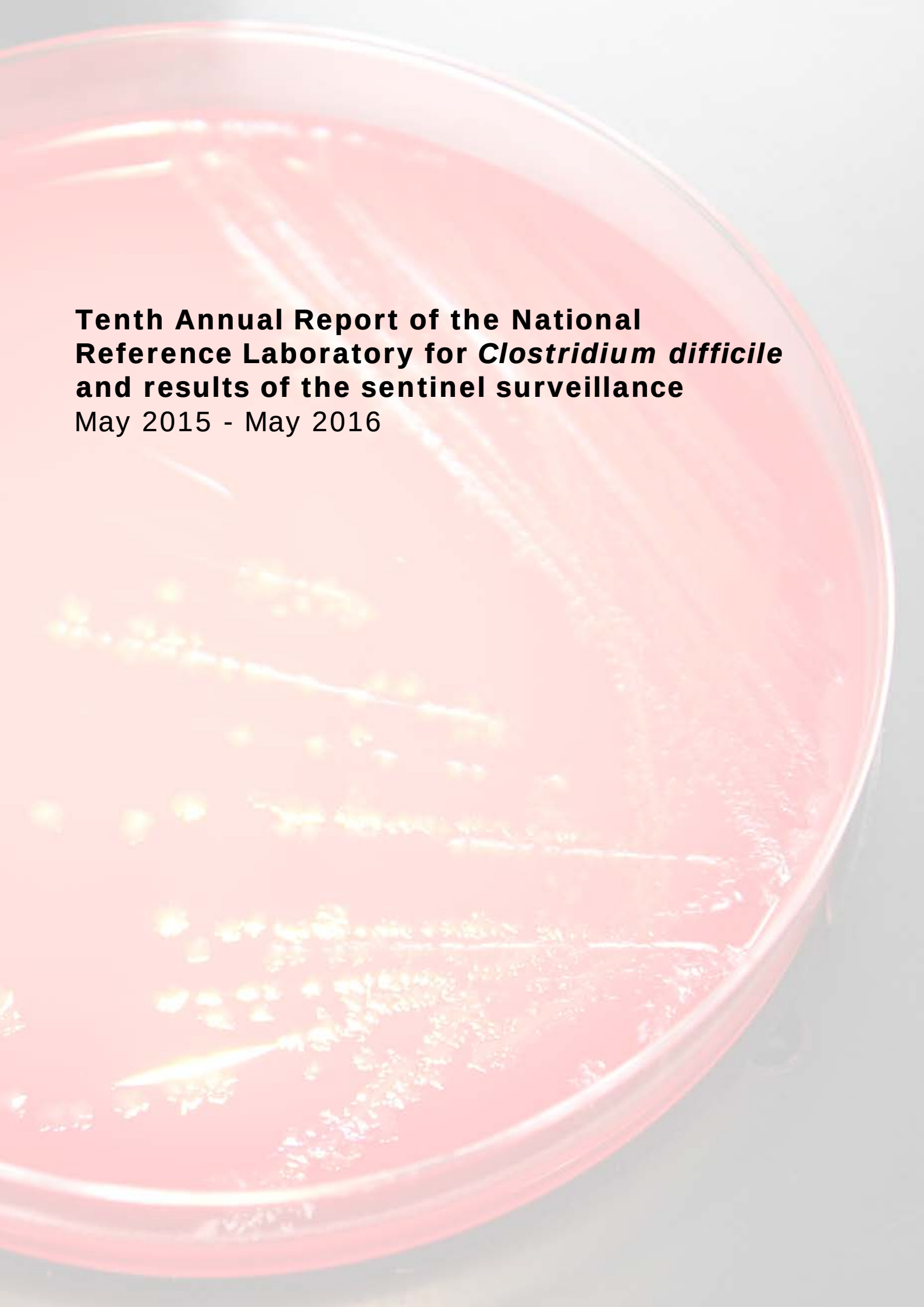


Tenth Annual Report of the National Reference Laboratory for *Clostridium difficile* and results of the sentinel surveillance

May 2015 - May 2016

Leiden University Medical Center, Department of Medical Microbiology

Drs. M.J.T Crobach, MD, PhD candidate
Drs. S.M. van Dorp, MD, PhD candidate
Ing. C. Harmanus, technician
Ing. I.M.J.G. Sanders, technician
Prof. dr. E.J. Kuijper, medical microbiologist

Centre for Infectious Disease Control (CIb), RIVM, Bilthoven

Dr. D.W. Notermans, medical microbiologist
Dr. Ir. S.C. de Greeff, epidemiologist
Ing. J. Alblas, datamanager
Prof. Dr. J.T. van Dissel, director

Acknowledgements

We thank R. Kewalapat, J. Hoegaerts-Goorman, M. Dittmar and T. Vreeswijk for their analytical support of the typing work. We also thank the administrative workers and technicians of the Department of Medical Microbiology of the LUMC for their contributions, and J.J.G. Schelfaut for coordination.

We sincerely thank the infection control personnel, medical microbiologists and laboratory technicians of all participating hospitals for their contribution.

Contact:
Drs. M.J.T. Crobach
M.J.T.Crobach@lumc.nl
Drs. S.M. van Dorp
svandorp@lumc.nl

Content

Key points

The National Reference Laboratory for *C. difficile*

- The National Reference Laboratory coordinates a sentinel surveillance program with 23 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 221 different PCR ribotypes.

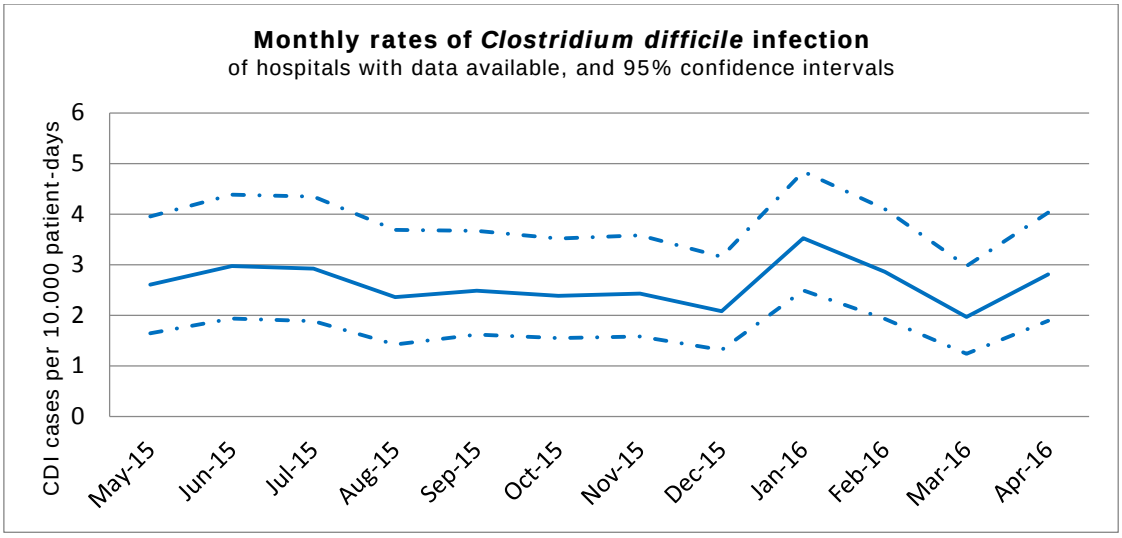


Figure 1. **Monthly rates of *Clostridium difficile* infection.** Data from 8 hospitals with monthly data available.

Results of the sentinel surveillance (May 2015- May 2016)

- A mean incidence rate of 3,09 CDI per 10.000 patient-days was found through sentinel surveillance (varying between hospitals from 0.69 to 5.88 CDI per 10.000 patient-days), similar to last years.
- The disease severity was reported for 845 out of 955 patients included in the surveillance; 21% had severe CDI. The 30-day outcome was reported for 752 patients; 1.3% of patients was admitted to the ICU due to CDI. None of the patients needed surgery because of CDI.
- 9.8% of the patients died within 30 days (n=74), of which 16 patients (2.1%) known to be contributable to CDI.
- No outbreaks were observed in the participating hospitals. In the Western part of the Netherlands a cluster comprising 5 patients was observed. This cluster was due to a newly identified ribotype, resembling ribotype 078.
- The most frequent encountered PCR ribotypes included ribotype 014/020 (17.0%), the closely related ribotypes 078 and 126 (12.9%), and ribotype 002 (7.2%). The proportion of ribotype 001 decreased from 26.5% to 3.4% over a period of seven years.
- Ribotype 027 was found in 1.2% of samples (0.7% during May 2014-May 2015)

Results of ad hoc typing (May 2015- May 2016)

- Fifteen healthcare facilities/laboratories sent 109 strains to the Reference Laboratory for ad hoc typing because of outbreaks, severe CDI cases, or for other reasons.
- Ribotype 027 was the predominant ribotype (20.0%), followed by ribotype 014 (19.0%).
- No 027 outbreak was observed during this period, but all 027 cases were clustered in the North-Western part of the Netherlands (where an 027 outbreak was observed during the period May 2014- May 2015)

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[1]), it is estimated that approximately 2900 hospitalized patients will develop CDI, and 61 patients succumb contributable to CDI annually. In these estimations, the impact of CDI in other healthcare facilities than hospitals is not included.

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.[2]

Diagnosis

The diagnosis of *C. difficile* infection (CDI) is most frequently based on clinical signs and symptoms in combination with laboratory tests. This year, a revision of the ESCMID guidelines on CDI diagnosis will be published (Crobach M et al, in press). 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 important if only test that detect the toxin-producing potential (i.e. toxin B PCR or toxigenic culture) are used instead of the detection of free toxins present is stools (i.e. 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 221 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 we know 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 and development of a National Donor Feces Bank (NDFB)

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] Despite antibiotic therapy, CDI recurrence is common. Fecal microbiota transplantation is proven to be very effective as treatment for recurrent CDI, likely by restoring the healthy gut microbiota.[10] 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 (http://www.ndfb.nl/). The aim of the NDFB is to make transplantation of carefully screened donor faeces easily available for patients in need of it. 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. The first fecal microbiota transplantation with a feces suspension from the NDFB was performed in May 2016.

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[11] and rapidly spread within Netherlands while causing major outbreaks.[12,13] Retrospectively, the rapid spread of the ribotype 027 strain across Northern-America and Europe has been attributed to its high level of fluoroquinolone resistance.[14] 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[12,15], which may reflect type-specific host susceptibility and/or an increased virulence of the strain.[16] Since mid-2006, the occurrence of ribotype 027 in the Netherlands has decreased significantly.[17] The CDI incidence rate has stabilised at 3 CDI cases per 10.000 patient-days.[18]

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, twenty-three 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).[18] This report is the tenth 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 2015 and May 1st 2016.

In 2015, ECDC has finalized a protocol for Europe-wide surveillance. A start-up data collection was performed in January 2016. The LUMC participated as a delegate of the Netherlands. In the coming year, this surveillance will be implemented further and the first European CDI report is expected in 2017. Participation of the Netherlands will be performed using the currently established network of sentinel CDI surveillance. The protocol is available at http://ecdc.europa.eu/en/publications/Publications/Clostridium-difficile-infections-surveillance-protocol-version-2.1.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=18) send strains to the laboratory of the Leiden University Medical Center. Other laboratories (n=5) send faecal samples.

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.[19,20] 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 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 June 21th 2016. 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 2016, the data from Jan-Apr 2015 were used as denominator. If no data were supplied by the hospital, data were acquired from jaarverslagenzorg.nl.[21] 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 12.1. 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*.[22] All *C. difficile* strains are further investigated by PCR-ribotyping.[3] The presence of *tcdA*, *tcdB* and binary toxin genes can be investigated by multiplex PCR on request.[23] Deletions in *tcdC* can be determined by PCR using in-house designed primers.

C. difficile Reference Library

The Reference Laboratory added 17 new ribotypes to the Reference Library in the prior year (types 022, 149, 155, 197, 247, 268, 341, 424, 454, 527, 533, 553, 608, 629, 722 and 760), and is now able to recognize 221 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, United 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.[19,20] 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 23 participating hospitals of the sentinel surveillance programme. Twenty-two of these hospitals already participated in the sentinel surveillance last year, and one hospital started surveillance in September 2015. Both university hospitals (n=6) and primary or secondary care hospitals (n=17) were included, distributed all over the Netherlands. The geographical location of the participating centres is displayed in Figure 1. One hospital was not able to submit clinical data for the last month of the surveillance period.

Figure 2. Participating hospitals of the sentinel surveillance by May 2016. University hospitals are depicted in orange, primary/secondary care hospitals are depicted in blue

Diagnostic testing

The primary diagnostic tests used by the participating hospitals to diagnose CDI are depicted in Table 3. By May 2016, 11/23 hospitals (48%) used an ESCMID recommended algorithm. Another 9 hospitals used NAAT (7 of these hospitals performed culture on NAAT-positive samples for confirmation and to have the isolates available for typing). Two hospitals used an enzyme immunoassay for toxins as a stand-alone test. Between May 2015 and May 2016, one hospital switched from Tox A/B EIA and culture to a (not recommended) algorithm. Another hospital switched from NAAT to a (recommended) algorithm. The mean percentage of *C. difficile* positive patients among all patients tested was 7% (range 4-10%; Table 3).

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 3.09 CDI per 10.000 patient-days (varying from 0.69 to 5.88 CDI per 10.000 patient-days), comparable to the incidence of 2.98 that was reported in 2014-2015.[18] Of hospitals that submitted monthly hospitals data (8 hospitals), the overall monthly rates were calculated over the year (see figure 1 in section Key points).

Demographical and clinical data

Demographical and clinical characteristics were collected from 955 patients included in the sentinel surveillance (Table 1). The mean age was 66 years, varying from 2 to 97 years. Of all patients, 2.1% (n=20) was younger than eighteen years old and 64.5% (n=617) was older than 65 years old. A total of 177 patients (21.0%) 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 752 patients. After 30 days, 668 patients (88.8%) had an

uncomplicated course of their CDI infection. On the other hand, 10 patients (1.3%) were admitted to the ICU as a consequence of CDI within 30 days, and 74 patients with CDI (9.8%) died. Sixteen deaths (2.1%) were due or contributable to CDI.

Table 1. Clinical characteristics and outcome of patients (n=955)

Patient characteristics and outcome	n / n[a]	%
Gender female	480/955	50,3%
Location of onset CDI		
hospital	478/954	50,1%
at home	402/954	42,1%
nursing home	31/954	3,3%
other health-care facility	43/954	4,5%
Hospital department		
Internal Medicine	188/476	39,5%
Surgery	85/476	17,9%
Lung diseases and TB	40/476	8,4%
Cardiology	27/476	5,7%
Gastroenterology	26/476	5,5%
Neurology	26/476	5,5%
ICU	17/476	3,6%
Geriatrics	16/476	3,4%
Pediatrics	9/476	1,9%
Other or unknown	42/476	8,9%
Antibiotics prior to CDI	530/808	65,6%
Recurrence	173/615	28,1%
Severe diarrhoea	177/845	21,0%
Pseudomembranous colitis	26/177	14,7%
Hypovolemia or hypo-albuminaemia	90/177	50,8%
Bloody diarrhoea	37/177	20,9%
Fever and leucocytosis	62/177	35,0%
Outcome		
Uncomplicated	668/752	88,8%
Surgery needed	0/752	0,0%
ICU admission needed	10/752	1,3%
Death, contributable to CDI	16/752	2,1%
Death, unrelated to CDI	51/752	6,8%
Death, cause unknown	7/752	0,9%

Submitted strains for PCR ribotyping

Of the 955 CDI patients detected though sentinel surveillance between May 1st 2015 and May 1st 2016, 761 *C. difficile* isolates could be PCR ribotyped and linked to the clinical data (80%). The most important reasons for missing data were the inability to culture *C. difficile* at the local laboratory (n=66) or the inability to type *C. difficile* at the National Reference laboratory (culture negative or negative for GluD PCR, n=76).

Circulating PCR ribotypes

Ribotype 014/020 (indistinguishable by ribotyping) was the most frequently found type, isolated in 129 of the 931 isolates (17.0%, 95% CI 14.3-19.6). The closely related ribotypes 078/126 were found in 98 isolates (12.9%; 95% CI 10.5-15.2), ribotype 002 in 55 isolates (7.2%; 95% CI 5.4-9.0), ribotype 001 in 26 isolates (3.4%; 95% CI 2.1-4.7) and ribotype 005 in 38 isolates (5.0%; 95% CI 3.5-6.5). Nine isolates were identified as ribotype 027 (1.2%; 95% CI 0.4-1.9) Of 50 isolates (6.5%, 95% CI 4.7-8.3) the PCR ribotype pattern was not recognized in our database, which is slightly lower than last year (13.3%; 95% CI 10.8-15.7). Thirty-seven different unknown ribotypes patterns were found, of which one was found ≥5 times (this ribotype was found in 5 clustered cases, see below). Ribotype 106, which is the most frequently found ribotype in the US,

was only found 2 times. 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 5.

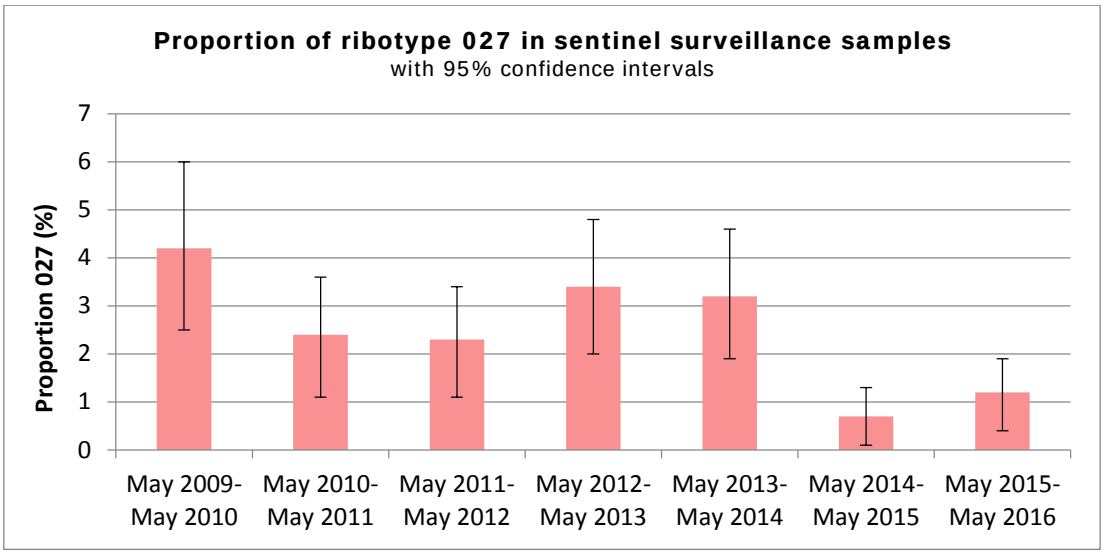


Figure 3. Proportion of ribotype 027 in sentinel surveillance samples.

Changes in circulating PCR ribotypes

The proportion of ribotype 001 continued to decrease compared to the previous years (2009-2010 95% CI 22.7-30.3, 2010-2011 95% CI 17.6-24.2, 2011-2012 95% CI 12.2-17.9, 2012-2013 95% CI 11.3-16.8, 2013-2014 95% CI 7.1-11.6, 2014-2015 95% CI 4.2-7.6). The proportion of ribotype 027 was slightly higher than last year, but lower than in the other preceding 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, 2014-2015 95% CI 0.1-1.3, figure 3). Ribotype 027 was found in five hospitals (5/23; 21.7%).

(Suspected) outbreaks in participating hospitals

A cluster of 5 epidemiologically and genetically linked cases was reported in a university hospital in the western part of the Netherlands. These cases occurred within a time period of 3 months on the same ward. An intriguing observation about this cluster is that these cases were due to an unknown ribotype, both unknown in our national database and international databases of known ribotypes. This new ribotype resembles the 078 ribotype (binary toxin positive, moxifloxacin resistant) and therefore, we should take into account the possible hypervirulent nature of this newly identified strain. (Crobach MJT and Voor in 't Holt AF et al, manuscript in preparation)

CDI among children

Data from the sentinel surveillance (period May 2009-May 2015) were used to describe the clinical and microbiological characteristics of CDI among hospitalized children. The number of annual pediatric CDIs was stable and ranged from 16 to 27 cases per year. The median age of children with CDI was 10 years and community-onset CDI was more common in children than in adults. Compared to adults, complication and mortality rates were lower. Ribtoype 265 was most frequently encountered in children (15%, 95% CI 9-24%), while this ribotype is rarely found in adults (1%, 95% CI 1-2%)). (van Dorp SM et al, manuscript accepted)

Table 2. Data from the sentinel surveillance for the period May 2015-May 2016 compared to the data from preceding years. The bottom line shows the number of ribotype 027 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
Incidence							
per 10.000 patient-days	2,7	2,8	2,9	2,9	2,9	3,0	3,1
Location of onset							
within healthcare facility	63%	73%	69%	63%	64%	59%	58%
at home	37%	27%	31%	37%	36%	41%	42%
Course and outcome							
Severe CDI	28%	20%	27%	25%	21%	24%	21%
Uncomplicated course	66%	86%	87%	88%	87%	86%	89%
Deaths contributable to CDI	4%	3%	4%	2%	3%	4%	2%
PCR ribotype 027							
Prevalence	4.2%	2.4%	2.3%	3.4%	3.2%	0.7%	1.2%
N reported 027 outbreaks-sentinel surveillance	1	1	0	1	0	0	0
N reported 027 outbreaks-ad hoc typing	2	2	1	2	5	1	0

Table 3. Number of patients included in the sentinel surveillance per hospital, and incidence data. Period: May 1st 2015 – May 1st 2016. 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)	% Positive	Months of participation	Patients		Monthly PD	Incidence per 10.000 PD	Incidence per 10.000 PD 2014-2015	Incidence difference
				N	%				
A	algorithm 3	4,3% (20/461)	12	3	0,3%	3599	0,69	2,17	-1,48
B	NAAT[1]	6,4% (195/3068)	12	23	2,4%	13592	1,41	2,25	-0,84
C	algorithm 2	NA	12	11	1,2%	6056	1,51	0,92	0,59
D	algorithm 3	3,9% (55/1411)	12	14	1,5%	5753	2,03	3,33	-1,30
E	NAAT[1]	7,9% (54/687)	12	25	2,6%	9964	2,09	3,13	-1,04
F	algorithm 1	8,4% (40/477)	12	18	1,9%	6353	2,36	2,76	-0,40
G	algorithm 4[3]	4,8% (133/2767)	12	35	3,7%	11858	2,46	3,42	-0,96
H	algorithm 3[5]	5,9% (252/4237)&	12	52	5,4%	17562	2,47	2,96	-0,49
I	Tox A/B EIA	5,0% (81/1634)	12	36	3,8%	12103	2,48	1,81	0,67
J	NAAT	7,6% (164/2162)	12	73	7,6%	24119	2,52	2,69	-0,17
K	algorithm 3	NA	12	52	5,4%	16010	2,71	1,89	0,82
L	NAAT[1]	6,9% (103/1498)	12	48	5,0%	13388	2,99	2,66	0,33
M	algorithm 2[2]	7,2% (63/881)	12	29	3,0%	7705	3,14	3,54	-0,40
N	NAAT[1]	6,7% (131/1941)	12	78	8,2%	20306	3,20	3,64	-0,44
O	algorithm 3	9,1% (133/1460)	12	55	5,8%	13860	3,31	3,38	-0,07
P	NAAT[4]	13,3% (211/1588)	12	63	6,6%	15300	3,43	3,10	0,33
Q	NAAT[1]	9,2% (99/1074)	11	47	4,9%	11977	3,57	3,29	0,28
R	Tox A/B EIA	9,0% (50/553)	12	38	4,0%	8403	3,77	2,90	0,87
S	algorithm 2	7,7% (141/1836)	12	68	7,1%	12745	4,45	3,99	0,46
T	algorithm 1	8,0% (104/1293)	8	57	6,0%	15521	4,59	NA	NA
U	NAAT[1]	8,6% (111/1297)	12	65	6,8%	11283	4,80	4,26	0,54
V	NAAT[1]	9,8% (101/1034)	12	43	4,5%	6940	5,16	5,56	-0,40
W	algorithm 3[5]	5,9% (252/4237)&	12	22	2,3%	3117	5,88	2,02	3,86
Total		**7,4%**	**271**	**955**	**100%**	**267914**	**3,09**	**2,98**	

A=not available; PD=patient-days; NAAT=Nucleic Acid Amplification Test; EIA= enzyme immunoassay
Algorithm 1: NAAT – Tox A/B EIA *(ESCMID recommended)*
Algorithm 2: GDH EIA- Tox A/B EIA (and in some hospitals also second confirmation with NAAT or toxigenic culture) *(ESCMID recommended)*
Algorithm 3: GDH & Tox A/B EIA (and in some hospitals confirmation with NAAT or toxigenic culture) *(ESCMID recommended)*
Algorithm 4: Tox A/B EIA – GDH EIA
[1] and culture of positive samples
[2] this hospital switched from NAAT to algorithm 2
[3] this hospital switched from Tox A/B EIA and culture to algorithm 4
[4] during weekends GDH & Tox A/B EIA, and confirmation of discrepant results by NAAT on the following day
[5] results from hospital H and W were combined (only concerning the diagnostic testing)

Table 4. The two most frequently found ribotypes per hospital, isolated amongst patients that were included in the sentinel surveillance. Period: 1st 2015 – May 1st 2016. If different PCR ribotypes were equally frequently found, the PCR ribotype with the lowest number is first reported. Ribotype 014/020 are indistinguishable by conventional ribotyping, and ribotype 078/126 can be hardly discriminated.

Hospital	N	%	type*	N**	%		N	%		N	%
A	3	0,3%	Isolates	3	100%	078/126	2	67%	014/020	1	33%
B	23	2,4%	Isolates	21	91%	002	5	24%	014/020	4	19%
C	11	1,2%	Isolates	3	27%	014/020	2	67%	078	1	33%
D	14	1,5%	Isolates	10	71%	039	2	20%	unknown	2	20%
E	25	2,6%	Isolates	21	84%	014/020	3	14%	unknown	3	14%
F	18	1,9%	Isolates	18	100%	014/020	3	17%	012	2	11%
G	35	3,7%	Isolates	34	97%	unknown	5	15%	002	3	9%
H	52	5,4%	Isolates	34	65%	014/020	6	18%	005	5	15%
I	36	3,8%	Isolates	31	86%	078/126	10	32%	014/020	8	26%
J	73	7,6%	Faeces	62	85%	unknown	10	16%	014/020	9	15%
K	52	5,4%	Isolates	37	71%	002	9	24%	014/020	6	16%
L	48	5,0%	Isolates	32	67%	014/020	4	13%	unknown	3	9%
M	29	3,0%	Faeces	27	93%	014/020	9	33%	078/126	4	15%
N	78	8,2%	Isolates	52	67%	014/020	10	19%	078/126	10	19%
O	55	5,8%	Isolates	51	93%	078/126	8	16%	002	5	10%
P	63	6,6%	Faeces	49	78%	014/020	12	24%	078	6	12%
Q	47	4,9%	Isolates	23	49%	014/020	5	22%	023	2	9%
R	38	4,0%	Faeces	21	55%	078/126	4	19%	unknown	4	19%
S	68	7,1%	Faeces	58	85%	014/020	9	16%	078126	5	9%
T	57	6,0%	Isolates	49	86%	014/020	8	16%	034	5	10%
U	65	6,8%	Isolates	63	97%	005	9	14%	014/020	9	14%
V	43	4,5%	Isolates	42	98%	078/126	11	26%	011	5	12%
W	22	2,3%	Isolates	20	91%	014/020	9	45%	070	4	20%
Total	**955**	**100%**		**761**	**80%**	**014/020**	**129**	**17,0%**	**078/126**	**98**	**12,9%**

*Dominant sample type send to LUMC; **Number of patients of whom a ribotyping results could be linked to the clinical data in OSIRIS.

Results of the ad hoc typing

Healthcare facilities and laboratories using the Reference Laboratory

In the period between May 1st 2015 and May 1st 2016, 15 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. However, Healthcare facility 12 inaccurately sent all *C. difficile* strains for typing without participating in the sentinel surveillance. In total, 109 samples were submitted for ad hoc PCR ribotyping.

Ad hoc ribotyping results

C. difficile could be cultured from 91.7% of the 109 submitted samples. The number of submitted isolates/samples and most common PCR ribotypes stratified per facility/laboratory, are demonstrated in table 5. Ribotype 027 was the most commonly found PCR ribotype (20.0%). Other frequently found ribotypes were 014 (19.0%), 001 (9.0%), 050 (5.0%) and 078 (4.0%). The percentage of ribotype 027 increased compared to last year, but varies in time: 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 decreased compared to last year (13% in 2014-2015). 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 5).

Outbreak investigation

This year, no outbreaks were officially reported to the National Reference Laboratory. All 027 isolates received by the Reference Laboratory isolates originated from 2 healthcare facilities in the North-Western part of the Netherlands (one of them serving several nursing homes in the region). Most of these isolates were submitted in the period May-August, as depicted in figure 4. This might indicate that in this regions, there were still problems with 027 infections.

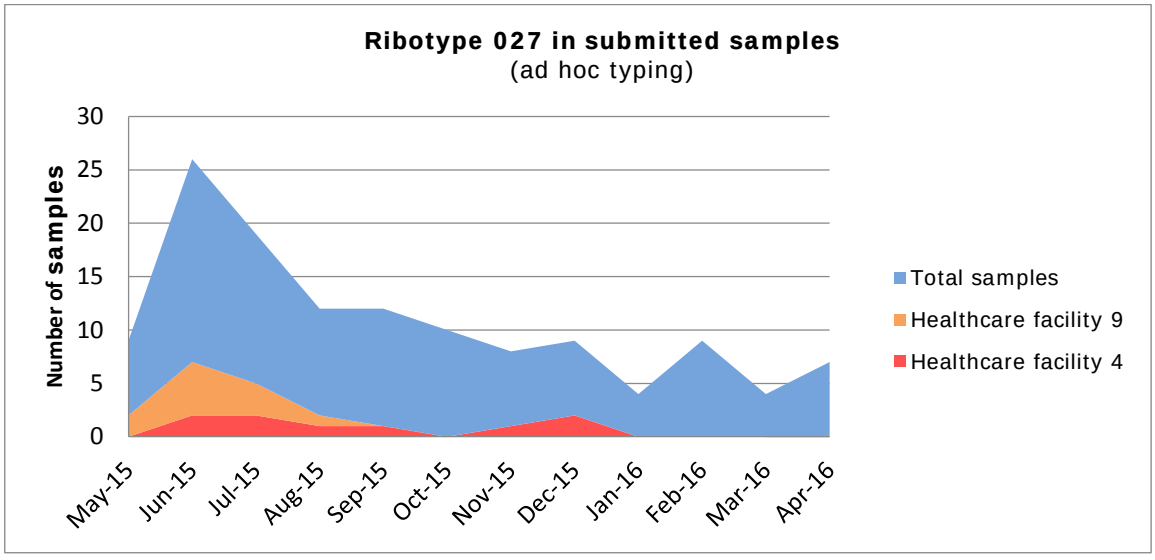
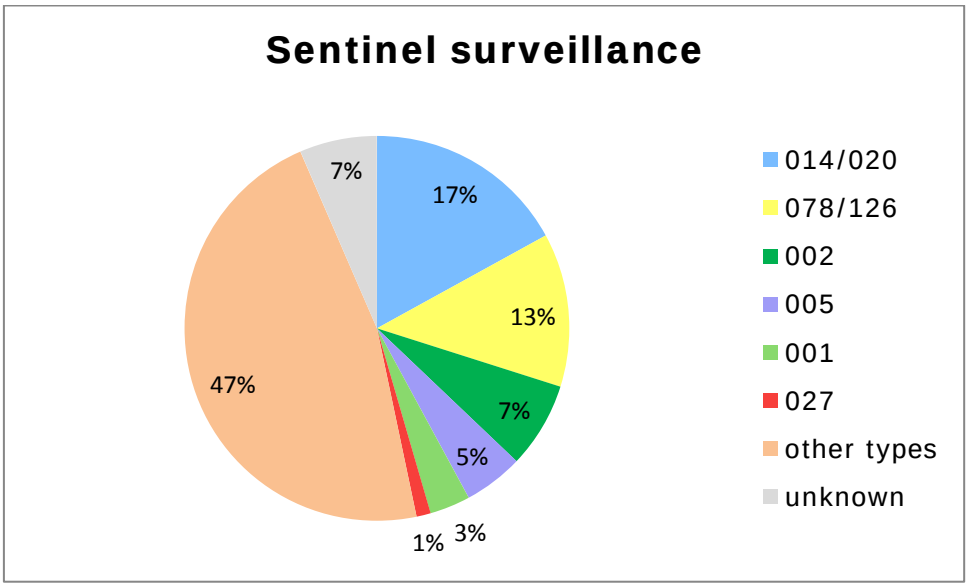


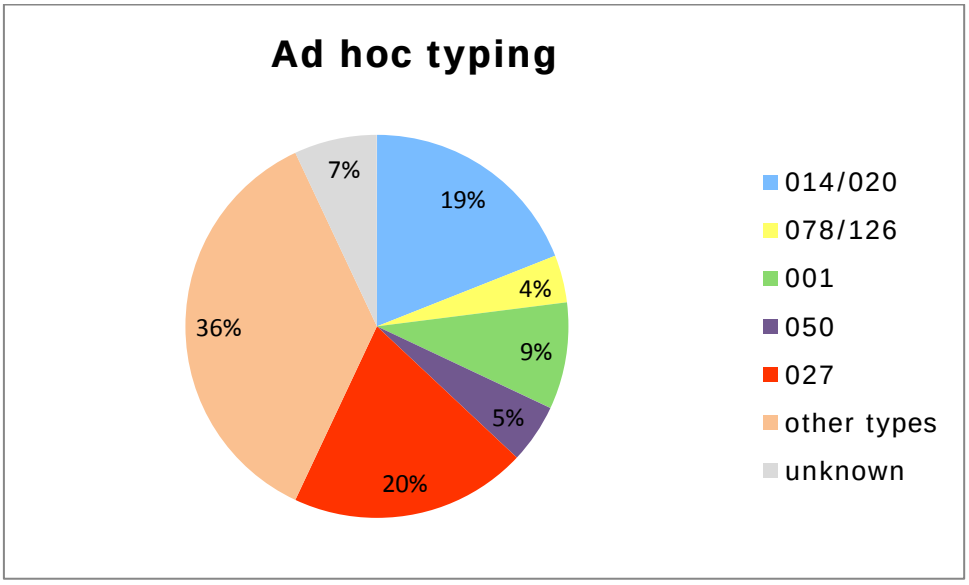
Figure 4. Proportion 027 in submitted samples. Total number of submitted samples per month depicted in blue. Samples from healthcare facility 9 depicted in orange. Samples from healthcare facility 4 depicted in blue.

Table 5. Results of the ad hoc typing.. Period: May 1st 2015 – May 1st 2016. If different PCR ribotypes were equally frequently found, the PCR ribotype with the lowest number is first reported. Ribotype 014/020 are indistinguishable by conventional ribotyping, and ribotype 078/126 can be hardly discriminated.

Laboratory / Healthcare facility	Samples		Sample type	*C. difficile*		Most common type			2nd most common type		
	N	%		N	%		N	%		N	%
1	1	1%	Faeces	0	0%	-	-	-	-	-	-
2	1	1%	Isolate	1	100%	023	1	100%	-	-	-
3	3	3%	Isolates	2	67%	014	1	50%	unknown	1	50%
4	33	30%	Isolates/faeces	32	97%	027	9	28%	014	7	22%
5	2	2%	Faeces	2	100%	003	1	50%	unknown	1	50%
6	1	1%	Faeces	1	100%	216	1	100%	-	-	-
7	1	1%	Isolate	1	100%	005	1	100%	-	-	-
8	1	1%	Faeces	1	100%	014	1	100%	-	-	-
9	32	29%	Isolates	31	97%	027	11	35%	014	5	16%
10	1	1%	Isolate	1	100%	014	1	100%	-	-	-
11	1	1%	Faeces	1	100%	078	1	100%	-	-	-
12	26	24%	Faeces	22	85%	014	3	14%	005	2	9%
13	2	2%	Faeces	1	50%	078	1	100%	-	-	-
14	2	2%	Isolates	2	100%	001	2	100%	-	-	-
15	2	2%	Isolates	2	100%	014	1	50%	052	1	50%
	109	**100%**		**100**	**91,7%**						

Figure 5. Proportions of five most frequent encountered PCR ribotypes and ribotype 027 for sentinel surveillance data, in comparison to ad hoc typing data. Period: May 1st 2015 – May 1st 2016. The category 'other types' consists of 83 different types in the sentinel surveillance data and 28 different PCR-ribotypes in the ad hoc typing data





Conclusions and recommendations

Sentinel surveillance:

- Although diverse CDI diagnostics are applied, almost half of hospitals participating in surveillance use optimal screening methods. A revised diagnostic guideline for CDI will be published later this year.
- The mean incidence rate of 3.09 CDI per 10.000 patient-days found though sentinel surveillance was comparable to the incidence rates (2.7-3.0) in 2009-2014.
- In comparison to previous years, there was no change in the occurrence of severe CDI (21.0%).
- No outbreaks were observed in hospitals participating in sentinel surveillance. Several small clusters were locally identified using the PCR ribotyping results.
- A cluster of 5 cases was reported in one hospital in the Western part of the country. This cluster was due to a formerly unknown ribotype, which resembles the 078 ribotype.
- The most frequent encountered PCR ribotypes included ribotype 014/020 (17%), the closely related ribotypes 078 and 126 (12.9%), and ribotype 002 (7.2%). The proportion of ribotype 001 decreased over the last seven years from 26.5 to 3.4%.
- Ribotype 027 was found in 1.2% (2014-2015: 0.7%). It was found in 5/23 (22%) participating hospitals.
- Extrapolating the data of sentinel surveillance to all hospitals in the Netherlands (with a total of 9.400.000 patient-days a year[1]), it is estimated that approximately 2900 hospitalized patients will develop CDI annually.
- We estimate that approximately 61 patients succumb contributable to CDI annually (CDI-related 30-day mortality of 2.1%). In these estimations, the impact of CDI in other healthcare facilities than hospitals was not included.

Ad hoc typing:

- Fifteen healthcare facilities/laboratories sent 109 strains to the Reference Laboratory for ad hoc typing because of outbreaks, severe CDI cases, or for other reasons.
- Ribotype 027 was the predominant ribotype, found in 20%, followed by ribotype 014/020 (19%) and ribotype 001 (9%).
- No 027 outbreaks were reported, but all 027 cases were clustered in the North-Western part of the country.

Output (May 2015-May 2016)

Completed PhD thesis

Wilco Knetsch. Molecular typing and evolution of *Clostridium difficile* (14-10-2015)

Participation of National Reference Laboratory in National and European activities

Granted Tender by ECDC: 'Supporting capacity building for surveillance of *Clostridium difficile* infections at European level' (2010-2015).

Euclid: Astellas sponsored study (2010-2015): European multi-centre prospective biannual point prevalence study of the incidence of *Clostridium difficile* Infection in patients with nosocomial diarrhoea (EUCLID).

ESCMID guidelines (2016-2017): Revision of guideline "Infection control measures to limit the spread of *Clostridium difficile*"

ESCMID guidelines (2014-2016): "Update of diagnostic guidance document for *Clostrdium difficile* infections."

Publications related to the reference laboratory

Smits WK, Lyras D, Lacy DB, Wilcox MH, Kuijper EJ. *Clostridium difficile* infection. Nat Rev Dis Primers. 2016;2:16020.

Pituch H, Obuch-Woszczatyński P, Lachowicz D, Wultańska D, Karpiński P, Młynarczyk G, van Dorp SM, Kuijper EJ; Polish *Clostridium difficile* Study Group. Hospital-based Clostridium difficile infection surveillance reveals high proportions of PCR ribotypes 027 and 176 in different areas of Poland, 2011 to 2013. Euro Surveill.2015;20(38).

Becker SL, Chatigre JK, Coulibaly JT, Mertens P, Bonfoh B, Herrmann M, Kuijper EJ, N'Goran EK, Utzinger J, von Müller L. Molecular and culture-based diagnosis of *Clostridium difficile* isolates from Côte d'Ivoire after prolonged storage at disrupted cold chain conditions. Trans R Soc Trop Med Hyg. 2015;109:660-8.

Troiano T, Harmanus C, Sanders IM, Pasquale V, Dumontet S, Capuano F, Romano V, Kuijper EJ. Toxigenic *Clostridium difficile* PCR ribotypes in edible marine bivalve molluscs in Italy. Int J Food Microbiol. 2015;208:30-4.

Nyc O, Krutova M, Liskova A, Matejkova J, Drabek J, Kuijper EJ. The emergence of *Clostridium difficile* PCR-ribotype 001 in Slovakia. Eur J Clin Microbiol Infect Dis. 2015;34:1701-8.

Fawley WN, Knetsch CW, MacCannell DR, Harmanus C, Du T, Mulvey MR, Paulick A, Anderson L, Kuijper EJ, Wilcox MH. Development and validation of an internationally-standardized, high-resolution capillary gel-based electrophoresis PCR-ribotyping protocol for *Clostridium diffi*cile. PLoS One. 2015;10(2):e0118150

Bouwknegt M, van Dorp S, Kuijper E. Burden of *Clostridium difficile* infection in the United States. N Engl J Med. 2015;372:2368.

Khanafer N, Voirin N, Barbut F, Kuijper E, Vanhems P. Hospital management of *Clostridium difficile* infection: a review of the literature. J Hosp Infect. 2015;90:91-101

Freeman J, Vernon J, Morris K, Nicholson S, Todhunter S, Longshaw C, Wilcox MH; Pan-European Longitudinal Surveillance of Antibiotic Resistance among Prevalent *Clostridium difficile* Ribotypes' Study Group. Pan-European longitudinal surveillance of antibiotic resistance among prevalent *Clostridium difficile* ribotypes. Clin Microbiol Infect. 2015 21:248.

Presentations and posters at congresses

26th European Congress of Clinical Microbiology and Infectious Diseases. Amsterdam, 9-12 April 2016

van Dorp S, Harmanus C, Sanders I, Dekkers OM, Knetsch C, Notermans D, de Greeff S, Kuijper EJ. Sentinel hospital surveillance of *Clostridium difficile* infections in the Netherlands during 2009-2015; ribotype 078/126 in relation to pig density. Poster P 0317.

van Dorp S, Smajlović E, Notermans D, de Greeff S, Kuijper EJ. Clinical and microbiological characteristics of *Clostridium difficile* infection among hospitalized children in the Netherlands; a six-year surveillance. Poster 676.

Knetsch C, Kumar N, Connor T, Corver J, Kuijper EJ, Lawley T. Global spread of multiple-antibiotic resistant *Clostridium difficile* between animals and humans. Oral presentation, abstract 2727.

Krutova M, Nyc O, Matejkova J, Tejkalova R, Petrlova K Hanslianova M, Balejova M, Paleckova V, Ryskova L, Petkov V, Vesela D, Havlinova L, Zamazalova D, Vagnerova I, Uhorskai P, Geigerova L, Jezek P, Kucharova A, Bartonikova N, Rumlerova M, Linhart P, Laskafeldova K, Hajna M, Kuijper EJ. Update on the molecular epidemiology of *Clostridium difficile* infections in the Czech Republic, 2015. Poster P1088.

Krutova M, Nyc O, Allerberger F, Wilcox MH, Kuijper EJ. Practical experiences with capillary electrophoresis ribotyping applied on Czech *Clostridium difficile* isolates collected over 3 years (2013-2015).Poster 0315.

Krutova M, Nyc O, MatejkovaJ, Kuijper EJ. Different distribution of *Clostridium difficile* PCR-ribotypes in acute care and long-term care wards of Czech hospitals. Poster P1089.

Stein K, Harmanus C, FitzGerald S, Roche F, Hennessy S, Drudy D, Kyne L, McDermott S, Burns K, Fitzpatrick F, Kuijper E, Fenelon L. Epidemiology of *Clostridium difficile* infection in Ireland 2014 & 2015. Poster P1086.

Carl Suetens, Pete Kinross on behalf of HAI(ECDIS)-CDI Network. News from CDI surveillance across Europe. Oral, S306.

Stein K, Egan S, Harmanus C, Kyne L, McDermott S, Kuijper E, Fitzpatrick F, FitzGerald S, Drudy, D, Fenelon L. The prevalence and PCR-ribotype distribution of *Clostridium difficile* in pigs in Ireland. Poster EV0277.

Voorjaarsvergadering van de Nederlandse Vereniging voor Medische Microbiologie (NVMM) en de Koninklijke Nederlandse Vereniging voor Microbiologie (KNVM). Papendal, 22 & 23 maart 2016

van Dorp S, Harmanus C, Sanders I, Dekkers O, Kampinga G, Knetsch W, Notermans D, de Greeff S, Kuijper EJ. Changing epidemiology of CDI in The Netherlands; six years of sentinel surveillance. Oral, O036

Crobach M, Terveer E, Verduin C, Vos M, Voss A, Kuijper EJ. The role of asymptomatic carriership for spread of CDI in hospitals. Oral, O037

Knetsch W, Connor T, Browne H, Kumar N, Harmanus C, Corver J, Kuijper EJ, Lawley T. Spread of multiple-antibiotic resistant *Clostridium difficile* type 078 between animals and humans at global scale. Poster, P066

3rd International Conference on Prevention & Infection Control (ICPIC 2015) 16-18 June 2015, Geneva.

Kuijper EJ, Maassen K, Crobach M. Time for active *Clostridium difficile* screening of asymptomatic high-risk carrier? Oral presentation.

Clostpath 9-Meeting, 7th – 11th Sept 2015. Freiburg, Germany

Kuijper EJ, van Dorp S, Notermans D. Epidemiology of *Clostridium difficile* infection in Europe.

38Th SOMED congress. Society for Microbiology Ecology and Diseases. 11-13 October 2015, Verona.

Kuijper EJ, van Dorp S, Notermans DW. Epidemiology and risk factors of *C. difficile* Infection in Europe inside and outside the healthcare institutions.

5rd International Clostridium difficile Symposium, 2015, Bled, Slovenia

Knetsch C, Connor T, Mutreja A, van Dorp S, Sanders I, Browne HP, Harris D, Lipman L, Keessen E, Corver J, Kuijper EJ, Lawley T. Whole genome sequencing reveals potential spread of Clsotrdium difficle between human and farm animals in The Netherlands, 2002 to 2011.

Invited presentations

11 January, 2016. Cursus Infectiepreventie, Utrecht. *Clostridium difficile* infecties.

October 28 and 28Th, 2015. Warschau. ZAKAŻENIA CLOSTRIDIUM DIFFICILE - PANDEMIA XXI WIEKU NOWOŚCI DIAGNOSTYKI, PROFILAKTYKI I TERAPII Z UDZIAŁEM MIĘDZYNARODOWYCH EKSPERTÓW. Ed J. Kuijper, S. van Dorp, and D. W. Notermans. The changing epidemiology of *Clostridium difficile* infections in Europe.

Participation and Organization of Workshops.

22 September 2015. Annual meeting of participants of CDI surveillance in The Netherlands

Postgraduate Technical Workshop (PGTW) "*Clostridium difficile*: Practical aspects of diagnostics, typing and comparative genomics". Maribor, Slovenia 2 – 4 September 2015

References

(1) Dutch Hospital Data. 2015. Available at: http://www.dutchhospitaldata.nl/

(2) Leffler DA, Lamont JT. Clostridium difficile infection. *N Engl J Med* 2015;372:1539-1548.

(3) Bidet P, Lalande V, Salauze B et al. Comparison of PCR-ribotyping, arbitrarily primed PCR, and pulsed-field gel electrophoresis for typing *Clostridium difficile*. *J Clin Microbiol* 2000;38:2484-2487.

(4) Curry SR, Muto CA, Schlackman JL et al. Use of Multilocus Vairable Number of Tandem Repeats Analysis Genotypinh to Determine the Role of Asymptomatic Carriers in *Clostridium difficile* Transmission. *CID* 2013;57(8):1094-102

(5) Eyre DW, Cule ML, Wilson DJ et al. Diverse sources of C. difficile infection identified on whole-genome sequencing. *N Engl J Med* 2013;369:1195-1205.

(6) Werkgroep Infectie Preventie. Infectiepreventieve maatregelen bij *Clostridium difficile*. Available at: http://www.rivm.nl/dsresource?objectid=rivmp:260520&type=org&disposition=inline&ns_nc=1v

(7) Khanafer N, Voirin N, Barbut F, Kuijper E, Vanhems P. Hospital management of Clostridium difficile infection: a review of the literature. *J Hosp Infect* 2015.

(8) Sethi AK, Al-Nassir WN, Nerandzic MM et al. Persistence of Skin Contamination and Environmental Sheding of Clostridium difficile during and after Treatment of *C. difficile* Infection. *Infect Control Hosp Epidemiol* 2010;31:21-27

(9) Debast SB, Bauer MP, Kuijper EJ et al. European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for Clostridium difficile infection. *Clin Microbiol Infect* 2014;20(suppl2):1-26

(10) van Nood E, Vrieze A, Nieuwdorp M et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med* 2013;368(5):407-15

(11) van SJ, Debast S, van KE, van den Berg R, Notermans D, Kuijper E. Isolation of Clostridium difficile ribotype 027, toxinotype III in the Netherlands after increase in C. difficile-associated diarrhoea. *Euro Surveill* 2005;10:E050714.

(12) Goorhuis A, van der Kooi T, Vaessen N et al. Spread and epidemiology of Clostridium difficile polymerase chain reaction ribotype 027/toxinotype III in The Netherlands. *Clin Infect Dis* 2007;45:695-703.

(13) Kuijper EJ, van den Berg RJ, Debast S et al. Clostridium difficile ribotype 027, toxinotype III, the Netherlands. *Emerg Infect Dis* 2006;12:827-830.

(14) He M, Miyajima F, Roberts P et al. Emergence and global spread of epidemic healthcare-associated *Clostridium difficile*. *Nat Genet* 2013;45:109-113.

(15) Hensgens MP, Goorhuis A, Dekkers OM, van Benthem BH, Kuijper EJ. All-cause and disease-specific mortality in hospitalized patients with Clostridium difficile infection: a multicenter cohort study. *Clin Infect Dis* 2013;56:1108-1116.

(16) Goorhuis A. Clostridium difficile Ribotype 027: An Intrinsically Virulent Strain, but Clinical Virulence Remains to Be Determined at the Bedside. *Clin Infect Dis* 2015.

(17) Hensgens MP, Goorhuis A, Notermans DW, van Benthem BH, Kuijper EJ. Decrease of hypervirulent *Clostridium difficile* PCR ribotype 027 in the Netherlands. *Euro Surveill* 2009;14.

(18) Documents and publications of the National Reference Laboratory for *C. difficile*. Available at: http://www.rivm.nl/Onderwerpen/C/Clostridium/Clostridium_difficile

(19) Kuijper EJ, Coignard B, Tüll P, ESCMID Study Group for Clostridium difficile, EU Member States, European Centre for Disease Prevention and Control. Emergence of *Clostridium difficile*-associated disease in North America and Europe. *Clin Microbiol Infect* 2006;12 Suppl 6:2-18.

(20) McDonald LC, Coignard B, Dubberke E, Song X, Horan T, Kutty PK. Recommendations for surveillance of *Clostridium difficile*-associated disease. *Infect Control Hosp Epidemiol* 2007;28:140-145.

(21) Jaarverslagen Zorg. www.jaarverslagenzorg.nl

(22) Paltansing S, van den Berg RJ, Guseinova RA, Visser CE, van der Vorm ER, Kuijper EJ. Characteristics and incidence of *Clostridium difficile*-associated disease in The Netherlands, 2005. *Clin Microbiol Infect* 2007;13:1058-1064.

(23) Persson S, Torpdahl M, Olsen KE. New multiplex PCR method for the detection of *Clostridium difficile* toxin A (tcdA) and toxin B (tcdB) and the binary toxin (cdtA/cdtB) genes applied to a Danish strain collection. *Clin Microbiol Infect* 2008;14:1057-1064.