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

May 2014 - May 2015

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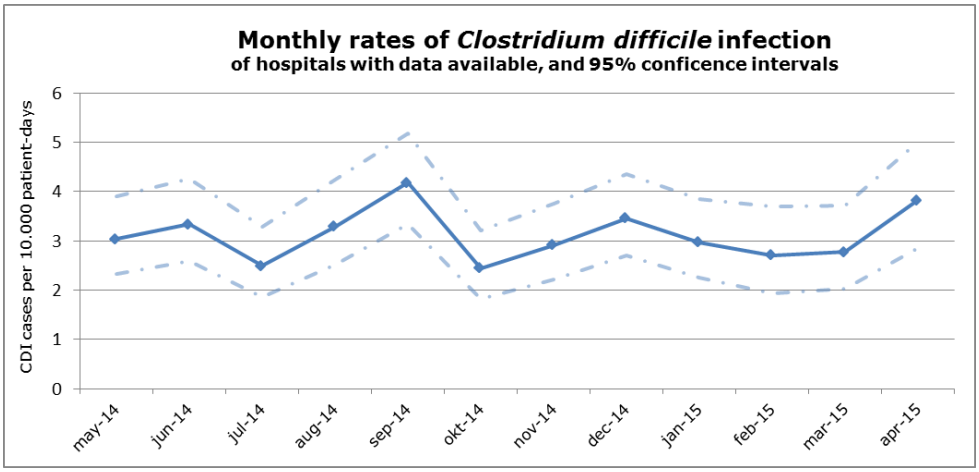
Content

Key points

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 204 different PCR ribotypes.



Results of the sentinel surveillance (May 2014- May 2015)

- A mean incidence rate of 2.98 CDI per 10.000 patient-days was found through sentinel surveillance (varying between hospitals from 0.92 to 5.56 CDI per 10.000 patient-days), similar to last years.
- The disease severity was reported for 806 out of 931 patients included in the surveillance; 24% had severe CDI. The 30-day outcome was reported for 715 patients; 0.1% and 1.1% of the patients needed surgery or were admitted to the ICU due to CDI, respectively.
- 12.6% of the patients died within 30 days (n=90), of which 31 patients (4.3%) known to be contributable to CDI.
- No outbreaks were observed in the participating hospitals.
- The most frequent encountered PCR ribotypes included ribotype 014/020 (16.3%), the closely related ribotypes 078 and 126 (12.9%), and ribotype 002 (7.1%). The proportion of ribotype 001 decreased from 26.5 to 5.9% over a period of six years.
- Ribotype 027 was less abundant than in the last five years (0.7% compared to 2.3-4.2%).

Results of ad hoc typing (May 2014- May 2015)

- Eleven healthcare facilities/laboratories sent 133 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 14.3%, followed by ribotype 078/126 (13.3%).
- One 027 outbreak was observed in the North-Western part of the Netherlands, whereas five 027 outbreaks were reported in 2013-2014. Some 027 cases in surrounding nursing homes were detected as well. An outbreak management team was able to rapidly control the outbreak, and the local public health service was consulted to coordinate *C. difficile*-related measures in surrounding nursing homes.

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 2800 hospitalized patients will develop CDI, and 120 patients succumb contributable to CDI annually. In these estimations, the impact of CDI in other healthcare facilities than hospitals is not included.
- We observed a substantial decrease of the burden of ribotype 027 compared to the prior year.

Introduction

Clostridium difficile infection

C. difficile is an anaerobic, spore-forming bacterium which can colonize the intestine of humans and animals. Pathogenic *C. difficile* strains 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]
The diagnosis of *C. difficile* infection (CDI) is based on clinical symptoms (diarrhoeal stools or toxic megacolon), and either a positive laboratory assay for *C. difficile* toxin A and/or B in stools or a toxin-producing *C. difficile* organism detected in stool via culture or other means, or endoscopy, or histopathology.[3] Additionally, *C. difficile* can be subtyped by PCR ribotyping using the type-dependent differences in profiles generated by PCR amplification of the intergenic spacer regions between the 23S and 16S rRNA genes.[4] The Reference Laboratory is now able to recognize 204 different PCR ribotypes.

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 firstly detected[5] and rapidly spread within Netherlands while causing major outbreaks[6;7]. Retrospectively, the rapid spread of the ribotype 027 strain across Northern-America and Europe has been attributed to its high level of fluoroquinolone resistance.[8] Besides, the presence of ribotype 027 was associated with unfavourable patient outcomes such as severe disease, mortality and recurrent CDI in comparison to other ribotypes[6;9], which may reflect type-specific host susceptibility and/or an increased virulence of the strain.[10] Since mid-2006, the occurrence of ribotype 027 in the Netherlands decreased significantly[11] and the incidence rate stabilised at 3 CDI cases per 10.000 patient-days[12].

Transmission and infection control

Transmission of *C. difficile* in the hospital setting is common, though one study from the UK suggested other sources of infection since 45% of the CDI cases was caused by a strain that was genetically different from previous CDI cases in the hospital.[13] Which other sources, perhaps asymptomatic carriers, contribute to CDI transmission is currently investigated in a new ZonMW supported multicentre study.
Yet, standard infection control precautions remain the bases for CDI prevention in the hospital setting. The national WIP guideline (July 2011) recommends application of contact precautions in combination with hospital cleaning and disinfection[14], though many Dutch hospitals do not enforce the use of high concentrations of chloride due to occupational health issues. In a recent review, environmental disinfection and antibiotic stewardship seem most effective to reduce CDI.[15]

The National Reference Laboratory

Soon after recognition of *C. difficile* ribotype 027 outbreaks in 2005, 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. This laboratory has facilities to type and characterize *C. difficile* isolates of patients with severe disease or when an outbreak is suspected for all microbiology laboratories in the Netherlands ('ad hoc typing services'). Additionally, the National Reference Laboratory initiated a sentinel surveillance programme in May 2009 to monitor the incidence of CDI in an endemic situation. Additionally, the programme aims to monitor (new) emerging strains of *C. difficile*. Currently, twenty-two acute care hospitals are participating in the sentinel surveillance programme voluntary. The results have been reported annually on the website of the National Institute for Public Health and the Environment (RIVM).[12]
This report is the ninth 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 2014 and May 1st 2015.

Aims and procedures of the sentinel surveillance

The national sentinel surveillance of *Clostridium difficile* infections has specific aims, additional to the aims of the reference laboratory:

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 all hospitalized CDI patients >2 years old with a positive toxin test for *C. difficile* in the surveillance. 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 toxin test that is used, is chosen by the local laboratory. Laboratories that culture *C. difficile* (n=17) 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 an 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.[3;16] 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. When 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, anonymised 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 16th of June 2015. To calculate incidence rates, we requested the participating hospitals to register their monthly number of admissions and number of admission-days. If not submitted, denominator data were subtracted from a website of the CIBG ('Centraal Informatiepunt Beroepen Gezondheidszorg') of the ministry of Health, Welfare and Sport[17]. 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.
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, SPSS for Windows software package version 20 and STATA/SE for Windows software package, version 12.1. Maps were created through FreeVectorMaps.com.

Aims and procedures of the ad hoc typing

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 a 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*.[18] All *C. difficile* strains are further investigated by PCR-ribotyping.[4] The presence of *tcdA*, *tcdB* and binary toxin genes can be investigated by multiplex PCR[19] on request. Deletions in *tcdC* can be determined by PCR using in-house designed primers.

C. difficile Reference Library

The Reference Laboratory added 30 new ribotypes to the Reference Library in the prior year (types 032, 034, 038, 041, 094, 140, 142, 144, 147, 158, 193, 209, 220, 237, 254, 269, 278, 288, 291, 513, 519, 572, 617, 626, 639, 643, 649, 650, 657, 668), and is now able to recognize 204 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

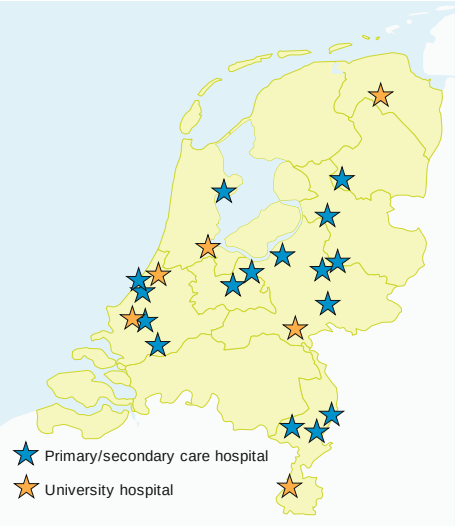
A standardized questionnaire is used to obtain information on patient's age and gender, the ward where CDI was acquired, clinical data, risk factors, antibiotic treatment in the month preceding a positive test and treatment outcomes. The definitions applied in this questionnaire are based on those proposed by the ECDC and the CDC.[3;16] 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. Compared to last year, one additional hospital (Hospital R) initiated surveillance in May 2014. All hospitals participated in the surveillance during the full twelve months, yet one hospital (Hospital G) was not able to submit clinical data for the last 3 months in time. Both university hospitals (n=6) and primary or secondary care hospitals (n=16) were included, distributed all over the Netherlands. The geographical location of the participating centres is displayed in Figure 1.

Figure 1. Participating hospitals of the sentinel surveillance by May 2015



Diagnostic testing

The primary diagnostic tests used by the participating hospitals to diagnose CDI are depicted in Table 3. By May 2015, 14% (n=3) of the hospitals used an enzyme immunoassay for toxins, 5% used an immunoassay for glutamate dehydrogenase (n=1), and 32% (n=7) a combination of both. Another 50% (n=11) used a Nucleic Acid Amplification Test (NAAT). Between May 2014 and May 2015, two hospitals switched from using toxin immunoassays to NAAT or immunoassays also targeting glutamate dehydrogenase. One hospital switched from a combined glutamate dehydrogenase and toxin immunoassay to NAAT, and one hospital did the opposite.
The mean percentage of *C. difficile* positive patients among all patients tested was 8% (range 5-13%; Table 3, data of 18 hospitals available).

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 2.98 CDI per 10.000 patient-days (varying from 0.92 to 5.56 CDI per 10.000 patient-days), similar to the incidence of 2.7-2.9 CDI per 10.000 patient-days that was reported in 2010-2014[12]. Of hospitals that submitted monthly hospitals data (15 hospitals), the overall monthly rates were calculated over the year (see figure in section Key points).

Demographical and clinical data

Demographical and clinical characteristics were collected from 931 patients included in the sentinel surveillance (Table 1). The mean age was 67 years (SD 18.5), varying from 3 to 98 years. 2.5% (n=23) of the patients was younger than eighteen years old. A total of 139 patients (24%) 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 714 patients. After 30 days, 615 patients (86.2%) had an uncomplicated course of their CDI infection. On the other hand, 8 patients (1.1%) were admitted to the ICU and 1 patient (0.1%)

needed surgery as a consequence of CDI within 30 days, and 90 patients with CDI (12.6%) died. Thirty-one deaths (4.3%) were due or contributable to CDI; four of these infections were caused by ribotype 002, four by ribotype 014/020, four by ribotype 078/126, and six by unknown ribotypes. The other infections were caused by ribotype 001, 003, 012, 015, 027, 045, 046, 050, 165. Of the remaining four patients, *C. difficile* could not (yet) be isolated and typed.

Table 1. Clinical characteristics and outcome of patients (n=931)

Patient characteristics and outcome	n/n[a]	%
Gender female	480/930	51.6%
Location of onset CDI		
Hospital	487/913	53.3%
At home	373/913	40.9%
Nursing home	24/913	2.6%
Other health-care facility	29/913	3.2%
Hospital department		
Internal Medicine	189/472	40.0%
Surgery	68/472	14.4%
Cardiology	33/472	7.0%
Gastroenterology	31/472	6.6%
ICU	28/472	5.9%
Lung diseases and TB	27/472	5.7%
Neurology	24/472	5.1%
Geriatrics	21/472	4.4%
Other	51/472	10.8%
Antibiotics prior to CDI	488/739	66.0%
Recurrence	139/598	23.2%
Severe CDI	193/806	23.9%
Pseudomembranous colitis	23/193	11.9%
Hypovolemia or hypo-albuminaemia	92/193	47.7%
Bloody diarrhoea	46/193	23.8%
Fever (>15,0 X 10^9/L) and leucocytosis (< 20 g/l)	71/193	36.8%
Outcome		
Uncomplicated	616/715	86.2%
Surgery needed	1/715	0.1%
ICU admission needed	8/715	1.1%
Death, contributable to CDI	31/715	4.3%
Death, unrelated to CDI	46/715	6.4%
Death, cause unknown	13/715	1.8%

[a]Number of patients with information available.

Submitted strains for PCR ribotyping

This year, we only report the PCR ribotyping results of patients that were included in the sentinel surveillance in contrast to the preceding year (when results of all submitted samples of participating hospitals were reported), for better adherence to the surveillance aims. Of the 931 CDI patients detected though sentinel surveillance between May 1st 2014 and May 1st 2015, 747 *C. difficile* isolates could be PCR ribotyped and linked to the clinical data (80.2%). The most important reasons for missing data were the inability to culture *C. difficile* at the local laboratory or the inability to type *C. difficile* at the National Reference laboratory (culture negative or negative for GluD PCR).

Circulating PCR ribotypes

Ribotype 014/020 (indistinguishable by ribotyping) was the most frequently found type, isolated in 122 of the 931 isolates (16.3%, 95% CI 13.7-19.0). The closely related ribotypes 078/126 were found in 96 isolates (12.9%; 95% CI 10.5-15.2), ribotype 002 in 53 isolates (7.1%; 95% CI 5.2-8.9), ribotype 001 in 44 isolates (5.9%; 95% CI 4.2-7.6) and ribotype 005 in 42 isolates (5.6%; 95% CI 4.0-7.3). Five isolates were identified as ribotype 027 (0.7%; 95% CI 0.1-1.3) and no 027-like PCR ribotypes were found. Of 99 isolates (13.3%, 95% CI 10.8-15.7) the PCR ribotype pattern was not recognized in our database, which is slightly higher than last year (8.9%; 95% CI 6.7-11.1). Seventy-seven different unknown ribotypes patterns were found, of which two were found ≥5 times. The results stratified per participating centre are displayed in Table 2. A pie-chart of the five most common ribotypes and ribotype 027 of patients included in the sentinel surveillance is illustrated in figure 4.

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). The proportion of ribotype 027 was lower than all 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). Ribotype 027 was found in three hospitals (Hospital F, N, and V, 3/22; 14%), that previously experienced 027 outbreaks. Ribotypes 081 (n=13), 295 (n=4; related to ribotypes 014/020) and 351 (n=4) were more often isolated than over the last years, though their absolute abundance is low.

(Suspected) outbreaks in participating hospitals

Between May 1st 2014 and May 1st 2015, no outbreaks were observed in participating hospitals of the sentinel surveillance. One hospital (Hospital U) contacted the National Reference Laboratory, because of a reintroduction of ribotype 001 that caused outbreaks in their hospital before. Multiple-Locus Variable number tandem repeat Analysis (MLVA) was used to study the relatedness of the reintroduced 001 strains with the previous outbreak strain. Three patients had an 001 strain that was clonally related to the previous outbreak strain, but four other patients had an 001 strain that could not be related to the previous outbreak strain or to one another. Because of the dispersion of the related 001 cases in time and the high-normal CDI incidence rate, it was not considered as an outbreak.

Figure 2. This MLVA minimum spanning tree depicts the spread of a 001 strain in hospital U in 2015 (shown in pink), and its relatedness to other 001 strains that were isolated in 2010 in the same hospital. Each circle contains 100% identical MLVA types, and the numbers in the circles/pies represent the number samples from this MLVA type per hospital/location. The lines between the circles indicate the summed tandem repeat difference (STRD) on a certain number of alleles (specified by the line style). The dark grey areas represent genetically related complexes (transmission likely) and light grey areas clonal complexes (direct transmission very likely).

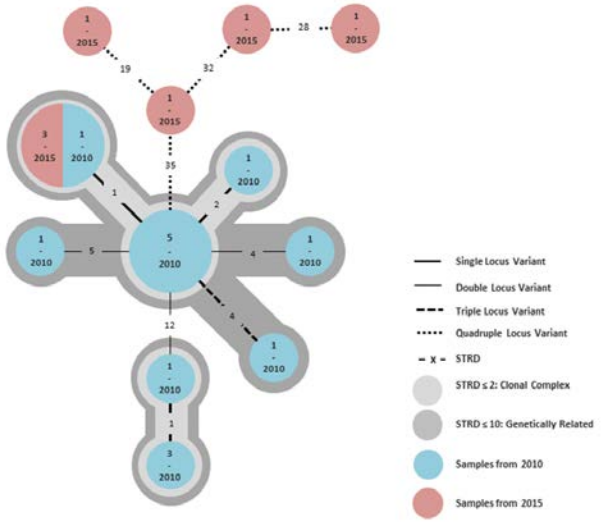


Table 2. The two most frequently found ribotypes per hospital, isolated amongst patients that were included in the sentinel surveillance between May 1st 2014 – May 1st 2015. If different PCR ribotypes were equally frequently found, the PCR ribotype with the lowest number is first reported. Ribotype 014/020 are indistinguishable by conventional ribotyping, and ribotype 078/126 can be hardly discriminated.

Hospital	Samples		Sample	*C. difficile*		Most common type			2nd most common type		
	N	%	type*	N	%		N	%		N	%
A	7	0.8%	Isolates	3	43%	010	1	33%	014/020	1	33%
B	27	2.9%	Isolates	22	81%	Various unknown types	6	27%	002	4	18%
C	37	4.0%	Isolates	32	86%	014/020	8	25%	002	7	22%
D	8	0.9%	Isolates	7	88%	023	2	29%	078/126	2	29%
E	10	1.1%	Isolates	9	90%	Various unknown types	3	33%	014/020	2	22%
F	37	3.8%	Isolates	35	95%	014/020	9	26%	078/126	7	20%
G	35	3.8%	Isolates	21	60%	078/126	5	24%	Various unknown types	3	14%
H	78	8.4%	Faeces	70	90%	014/020	15	21%	078/126	14	20%
I	21	2.3%	Isolates	17	81%	014/020	6	35%	012	2	12%
J	30	3.2%	Faeces	17	57%	015	3	18%	011	2	12%
K	63	6.8%	Isolates	45	71%	014/020	9	20%	002	7	16%
L	58	6.2%	Faeces	44	76%	Various unknown types	10	23%	005	5	11%
M	38	4.1%	Isolates	32	84%	Various unknown types	7	22%	014/020	5	16%
N	52	5.6%	Isolates	30	58%	014/020	6	20%	078/126	6	20%
O	28	3.0%	Isolates	22	79%	014/020	3	14%	023	3	14%
P	57	6.1%	Isolates	52	91%	014/020	10	19%	078/126	8	15%
Q	51	5.5%	Isolates	50	98%	Various unknown types	12	24%	078/126	9	18%
R	33	3.5%	Faeces	29	88%	078/126	7	24%	001	4	14%
S	92	10.0%	Isolates	65	71%	014/020	10	15%	Various unknown types	10	15%
T	61	6.6%	Faeces	50	82%	001	8	16%	078/126	8	16%
U	59	6.3%	Isolates	48	81%	014/020	7	15%	005	6	13%
V	49	5.3%	Isolates	47	96%	014/020	9	19%	078/126	8	17%
Total	**931**	**100%**		**747**	**80%**	**014/020**	**122**	**16.3%**	**078/126**	**96**	**12.9%**

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

Table 3. Number of patients included in the sentinel surveillance per hospital, and incidence data. Period: May 1st 2014 – May 1st 2015. The primer diagnostic test for CDI is shown per hospital; if the applied diagnostic test changed during the surveillance period, two subsequent tests are reported. The incidence per 10.000 patient-days is compared the results of the previous annual report, demonstrated as an incidence difference.

Hospital	Diagnostic test	% Positive	Months of participation	Patients N	Patients %	Monthly PD	Incidence per 10.000 PD	Incidence per 10.000 PD 2013-2014	Incidence difference
A	EIA[4]	13% (47/353)	12	7	0.8%	6347	0.92	2.23	-1.31
B	EIA[1]	10% (140/1418)	12	27	2.9%	12397	1.81	2.65	-0.83
C	EIA[3]	*NA*	12	37	4.0%	16355	1.89	2.38	-0.49
D	EIA[1]; EIA[3]	4% (6/153)*	12	8	0.9%	3300	2.02	0.89	1.13
E	EIA[3]	5% (24/465)	12	10	1.1%	3849	2.17	1.34	0.82
F	NAAT	6% (160/2724)	12	37	4.0%	13696	2.25	5.74	-3.49
G	EIA[1]; NAAT	*NA*	9	35	3.8%	14624	2.66	3.50	-0.84
H	NAAT	6% (116/1859)	12	78	8.4%	24153	2.69	1.75	0.94
I	NAAT	9% (31/341)	12	21	2.3%	6345	2.76	3.44	-0.68
J	EIA[2]	5% (54/1172)	12	30	3.2%	8623	2.90	2.49	0.41
K	EIA[3]	6% (89/1488)	12	63	6.8%	17752	2.96	3.35	-0.39
L	NAAT	13% (78/621)	12	58	6.2%	15600	3.10	2.07	1.02
M	EIA[3]; NAAT	10% (54/564)	12	38	4.1%	10118	3.13	4.21	-1.09
N	NAAT	10% (111/1073)	12	52	5.6%	13183	3.29	2.83	0.46
O	EIA[3]	*NA*	12	28	3.0%	7016	3.33	1.82	1.50
P	NAAT; EIA[3]	8% (83/1061)	12	57	6.1%	14055	3.38	2.54	0.84
Q	EIA[1]	*NA*	12	51	5.5%	12412	3.42	3.74	-0.31
R	NAAT	9% (69/761)	12	33	3.5%	7760	3.54	*NA*	*NA*
S	NAAT	7% (145/1959)	12	93	10.0%	21264	3.64	3.40	0,25
T	EIA[3]	12% (150/1276)	12	61	6.5%	12725	3.99	2.30	1.69
U	NAAT	7% (62/941)	12	59	6.3%	11549	4.26	4.67	-0.41
V	NAAT	9% (66/776)	12	49	5.3%	7339	5.56	5.57	-0.01
Total		**8%**	**261**	**932**	**100%**	**260461**	**2.98**		

NA=not available; PD=patient-days; NAAT=Nucleic Acid Amplification Test; EIA1=Toxin assay (Vidas®); EIA2=Toxin assay (Immunocard®); EIA3= GDH and toxin assay (C. diff Quik Chek Complete® or Liaison®); EIA4=GDH assay (RidaQuick®) *Data as of the 7th of January 2015 missing.

Results of the ad hoc typing

Healthcare facilities and laboratories using the Reference Laboratory

In the period between May 1st 2014 and May 1st 2015, 11 healthcare facilities and laboratories in the Netherlands sent samples to the Reference Laboratory in Leiden for ad hoc typing (Table 4). The samples were sent for other reasons than for sentinel surveillance, such as severe CDI or suspicion of an outbreak (Healthcare facility 1/Laboratory 3). However, Healthcare facility 2 inaccurately sent all *C. difficile* strains for typing without participating in the sentinel surveillance. In total, 133 samples were submitted for ad hoc PCR ribotyping.

Ad hoc ribotyping results

Of the 133 samples submitted, 85.0% contained *C. difficile.* The number of submitted isolates and most common PCR ribotypes stratified per facility/laboratory, are demonstrated in table 4. Ribotype 027 was the most commonly found PCR ribotype (14.2%), followed by ribotype 078/126 (13.3%), ribotype 014/020 (12.4%), and ribotype 001 (8.8%). The percentage of ribotype 027 decreased compared to last year, but varies in time: 32% in 2013-2014, 20% in 2012-2013, 15% in 2011-2012, 26% in 2010-2011, and 4% in 2009-2010. 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 4).

Outbreak investigation

In the period of May 2014-May 2015, we reported a remarkable high number of 027 outbreaks (n=5). All of the healthcare facilities involved were able to control the outbreaks by additional infection control measures i.e. improvement of basic hygiene, enhanced contact precautions and restricted use of fluoroquinolones. Since 1st of May 2014, one outbreak was observed by the National Reference Laboratory. The outbreak started on a surgery department of a primary care hospital located in the North-Western part of the Netherlands by the end of 2014 (Healthcare facility 1). In total, eleven cases developed CDI caused by ribotype 027. Multiple-Locus Variable number tandem repeat Analysis (MLVA) demonstrated clonal transmission of an 027 strain, that was clonally related to 027 strains that were isolated in the same hospital in 2013 (Figure 4). Further, the 027 strain was genetically related to one of the strains from a major 027 outbreak in the same region in 2013-2014. The outbreak has been controlled by enhanced hospital cleaning and surveillance. Other microbiologists have been informed of these findings in the 'signaleringsoverleg' on the 19th of February 2015.

Figure 3: This MLVA minimum spanning tree depicts the clonal spread of a 027 strain in healthcare facility 1 (shown in purple) in 2015. Further, its relatedness is shown to other 027 strains that were isolated in 2013 in the same healthcare facility, three former 027 outbreak strains from healthcare facilities in the same region (2013; shown in pink and green), and two 027 strains that were found by sentinel surveillance in another part of the country (shown in blue). Each circle contains 100% identical MLVA types, and the numbers in the circles/pies represent the number samples from this MLVA type per hospital/location. The lines between the circles indicate the summed tandem repeat difference (STRD) on a certain number of alleles (specified by the line style). The dark grey areas represent genetically related complexes (transmission likely) and light grey areas clonal complexes (direct transmission very likely).

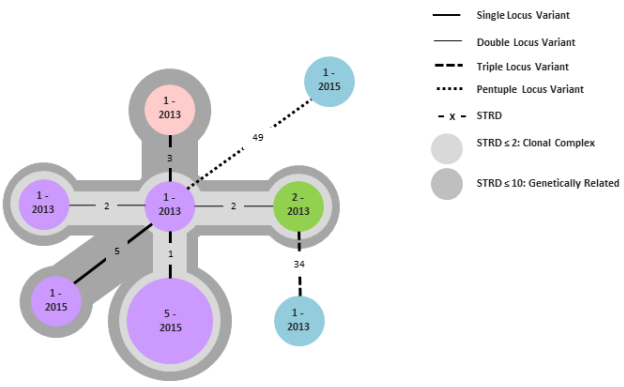
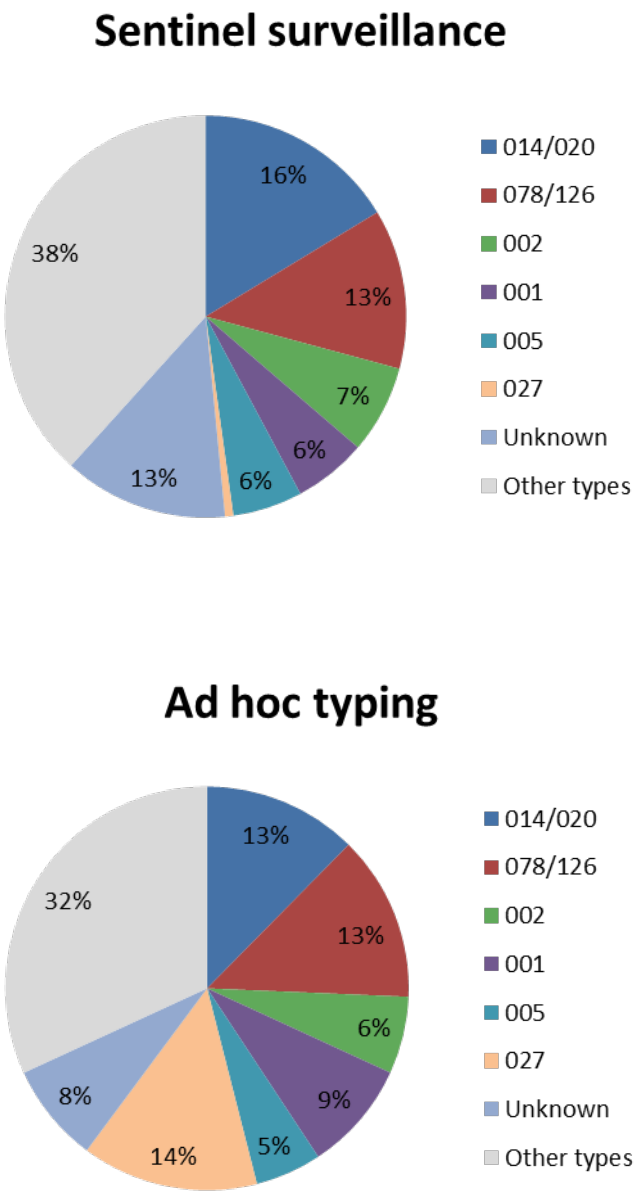


Table 4. Number of isolates sent to the Reference Laboratory for ad hoc typing per location. Period: May 1st 2014 – May 1st 2015. If different PCR ribotypes were equally frequently found, the PCR ribotype with the lowest number is first reported. Ribotype 014/020 are indistinguishable by conventional ribotyping, and ribotype 078/126 can be hardly discriminated.

Laboratory / Healthcare facility	Samples[b]		Sample	*C. difficile*		Most common type			2nd most common type		
	N	%	type	N	%		N	%		N	%
1	55	41%	Isolates	47	85%	027	9	19%	078/126	7	15%
2	33	25%	Isolates	26	79%	078/126	5	19%	001	4	15%
3[a]	30	23%	Isolates	29	97%	014/020	6	21%	027	5	17%
4	6	5%	Isolates/faeces	3	50%	014/020	1	33%	078/126	1	33%
5	2	2%	Isolates	2	100%	002	1	50%	078/126	1	50%
6	2	2%	Isolates	2	100%	002	1	50%	070	1	50%
7	1	1%	Isolate	1	100%	Unknown	1	100%	-	-	
8	1	1%	Faeces	1	100%	Unknown	1	100%	-	-	
9	1	1%	Faeces	0	0%	-	-		-	-	
10	1	1%	Faeces	1	100%	026	1	100%	-	-	
11	1	1%	Isolate	1	100%	027	1	100%	-	-	
	133	**100%**		**113**	**85.0%**	**027**	**16**	**14.2%**	**078/126**	**15**	**13.3%**

[a]Serving several nursing homes; [b]Isolates and faecal samples

Figure 4. 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 2014 – May 1st 2015. The category 'other types' consists of 63 different types in the sentinel surveillance data and 23 different PCR-ribotypes in the ad hoc typing data.



Conclusions and recommendations

- Although diverse CDI diagnostics are applied, most 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 2.98 CDI per 10.000 patient-days found though sentinel surveillance was similar to the 2.7-2.9 CDI per 10.000 patient-days as in 2009-2014.
- In comparison to previous years, there were no changes in the occurrence of severe CDI (24%), and CDI-related 30-day mortality (4.3%).
- No outbreaks were observed in hospitals participating in sentinel surveillance.
- The most frequent encountered PCR ribotypes included ribotype 014/020 (16.3%), the closely related ribotypes 078 and 126 (12.9%), and ribotype 002 (7.1%). The proportion of ribotype 001 decreased over the last six years from 26.5 to 5.9%.
- Ribotype 027 was less abundant than in the last six years (0.7% compared to 2.3-4.2%) in hospitals participating in sentinel surveillance.
- Eleven healthcare facilities/laboratories sent 133 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 14.2%, followed by ribotype 078/126 (13.3%) and ribotype 014/020 (12.4%).
- One 027 outbreak was observed in the North-Western part of the Netherlands, whereas last year five 027 outbreaks were reported. The 027 outbreak strain was clonally related to 027 strains that were isolated in the same hospital in 2013, and genetically related to 027 outbreak strains isolated in the same region in 2013-2014. Some 027 cases in surrounding nursing homes were detected as well.
- 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 2800 hospitalized patients will develop CDI annually.
- We estimate that approximately 120 patients succumb contributable to CDI annually (CDI-related 30-day mortality of 4.3%). In these estimations, the impact of CDI in other healthcare facilities than hospitals was not included.
- After last year's re-emergence of ribotype 027 in several Dutch healthcare facilities, we detected a substantial lower burden of 027 this year (n=24) compared to last year.

Output (May 2014-May 2015)

Completed PhD thesis

Dennis Bakker. Molecular characterization of pathogenic *Clostridium difficile* strains. 5th of November, 2014.

Martijn Bauer. *Clostridium difficile* infections: Epidemiology, complications and recurrences. 22nd of October 2014.

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' (2010-2014).

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

Publications related to the reference laboratory

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Presentations and posters

25th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID 2015), Copenhagen, 25 april-28 april 2015.

O266 Survey of diagnostic and typing capacity for *Clostridium difficile* infection across Europe in 2011 and 2014; van Dorp, Sofie; Notermans, Daan; Alblas, Jeroen; Barbut, Frédéric; Gastmeier, Petra; Virolainen-Julkunen, Anni; Nagy, Elisabeth; Spigaglia, Patrizia; Ivanova, Kate; Fitzpatrick, Fidelma; Morris, Trefor; Wilcox, Mark; Kinross, Pete; Suetens, Carl; Kuijper, Ed; ECDIS-Net, on behalf of all participants;

O267 Transmissibility of *C. difficile* without contact isolation: results from a prospective observational study. Widmer, Andreas; Frei, Reno; Lawley, Trevor; Anne, Stranden; Kuijper, Ed; Knetsch, Cornelis; Tschudin-Sutter, Sarah;

O269 Update of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) diagnostic guidance document for *Clostridium difficile* infection (CDI). Crobach, Monique Jacqueline Theresia; Planche, Timothy; Dekkers, Olaf; Terveer, Liz; Eckert, Catherine; Barbut, Frederic; Wilcox, Mark; Kuijper, Ed;

O166 Global spread of *Clostridium difficile* type 078 and its close relatives between animals and humans. Knetsch, Wilco; Kuijper, Ed; Lawley, Trevor; International studygroup.

P0793 Application of a microchip electrophoresis system for PCR ribotyping of *Clostridium difficile.* Sanders, Ingrid; Kraakman, Margriet; Harmanus, Celine; Claas, Eric; Kuijper, Ed J;

P0582 Sentinel surveillance for *Clostridium difficile* infections in The Netherlands reveals stable incidence. van Dorp, Sofie Maria; Notermans, Daan; de Greeff, Sabine; Harmanus, Céline; Kuijper, Ed;

Invited presentations

Kongress für Infektionskrankheiten und Tropenmedizin, 25. - 28. Juni 2014, Köln, Gürzenich.
E.J. Kuijper, W. Knetsch , S. van Dorp and D.W Notermans . *Clostridium difficile* infections in animals and humans; trends and expectations.

Royal College of Physicans of Edinburgh symposium. Culture change: the modern diagnosis & management of bacterial infection Friday 6 June 2014.
SYDNEY WATSON SMITH LECTURE (Chair: Professor Derek Bell, President, Royal College of Physicians of Edinburgh).
Ed J. Kuijper, Marjolein Hensgens and Josbert Keller. New aspects of Clostridium difficile infections; the role of the community and the microbiota

Expert Lecture on C Diff Infection Current and Emerging Trends on Tuesday 10th June 2014 in the Dun Library, Royal College of Physicians of Ireland, for Royal College of Physicians in Dublin, Ireland.
Ed J. Kuijper, Sofie van Dorp, Marjolein Hensgens and Daan Notermans. *Clostridium difficile* infections; current and emerging trends.

8 Oktober 2014, Veldhoven. Cursorisch onderwijs in maag-darm-leverziekten.
Ed J. Kuijper. Diarree na antibiotica

Healthcare Infection Society, Lyon, 16-18 November 2014.
S.M. van Dorp, M.P.M. Hensgens, E.J. Kuijper. Prediction rules and clinical scores of CDI
Ed J. Kuijper, Sofie van Dorp, Marjolein Hensgens and Daan Notermans. *Clostridium difficile* infections; current and emerging trends.

Facultad de Veterinaria, Universidad Complutense, Madrid, 28 November 2014.
Symposium "Epidemiological surveillance of *Clostridium difficile* in animal health"
Ed J. Kuijper, Sofie van Dorp, Marjolein Hensgens and Daan Notermans. Molecular epidemiology of *Clostridium difficile*

16 Januari 2015. Landgoed de Rosep, Oisterwijk. Cursus Infectiepreventie 2015.
Ed J. Kuijper. *Clostridum difficile* infecties.

"5° AMIT International Congress", Milan - March 12th and 13th, 2015
Ed J. Kuijper, S. van Dorp, D. Notermans and J. Keller. Evolution in the management of *Clostridium difficile* infections.

ISICEM 2015, 35TH The International Symposium on Intensive Care and Emergency Medicine Brussels, Belgium – 17 – 20 March 2015
Edward Kuijper et al. Challenges for Preventing Recurrent *C. difficile* Infections

Hot Topics in Microbiology 23–26 April, 2015, Slovakia, 23-26 April 2015.
Ed J. Kuijper, S. van Dorp and D.W Notermans. Update on *Clostridium difficile* epidemiology and treatment.

5rd International Clostridium difficile Symposium, 19-21 May 2015, Bled, Slovenia.
W. Knetsch, et al. Whole genome sequencing reveals potential spread of *Clostridium difficile* between humans and farm animals in the Netherlands, 2002 to 2011.
K. Stein et al. Analysis of the epidemiology of *Clostridium difficile* infection in Ireland, 2014

Organization of Workshops and congress sessions

The 9th **Healthcare Infection Society International Conference** 2014, Lyon, 16 – 18 November 2014: 2-hours Session: Current challenges with *Clostridium difficile*

Session at **ECCMID 2015**; News from basic science for clinical application in *Clostridium difficle* infections.

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