Eleventh 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. Last year, 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 249 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 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] 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.[10] Fecal microbiota transplantation is proven to be very effective as treatment for recurrent CDI, likely by restoring the healthy gut microbiota.[11] 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.[12] 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=40 fecal microbiota transplantations with a feces suspension from the NDFB were performed in the period May 2016-May 2017.

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[13] and rapidly spread within Netherlands while causing major outbreaks.[14,15] Retrospectively, the rapid spread of the ribotype 027 strain across Northern-America and Europe has been attributed to its high level of fluoroquinolone resistance.[16] 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[14,17], which may reflect type-specific host susceptibility and/or an increased virulence of the strain.[18] Since mid-2006, the occurrence of ribotype 027 in the Netherlands has decreased significantly.[19] The CDI incidence rate has stabilised at 3 CDI cases per 10.000 patient-days.[20]

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, 24 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).[20] This report is the eleventh 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 2016 and May 1st 2017.

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=19) 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.[21,22] 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 July 4th 2017. 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 2017, the data from Jan-Apr 2016 were used as denominator. If no data were supplied by the hospital, data were acquired from jaarverslagenzorg.nl.[23] 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, 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

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.[25] Deletions in *tcdC* can be determined by PCR using in-house designed primers.

C. difficile Reference Library

The Reference Laboratory added 27 new ribotypes to the Reference Library in the prior year, and is now able to recognize 249 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.[21,22] 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 24 participating hospitals of the sentinel surveillance programme. Both university hospitals (n=6) and primary or secondary care hospitals (n=18) 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 2017. 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 2 and Figure 2. By May 2017, 13/24 hospitals (54%) used an ESCMID recommended algorithm. Another 8 hospitals (33%) 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. Seven of the 8 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 and one hospital used a not-recommended algorithm. According to the latest ESCMID guidelines all submitted unformed stool samples from patients 3 years or older should be tested for CDI. By May 2017, only 4/24 hospitals (17%) complied with this recommendation. Eight out of 24 hospitals (33%) 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 (29%) 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 7.1% (range 3.1-13.8%; Table 3).

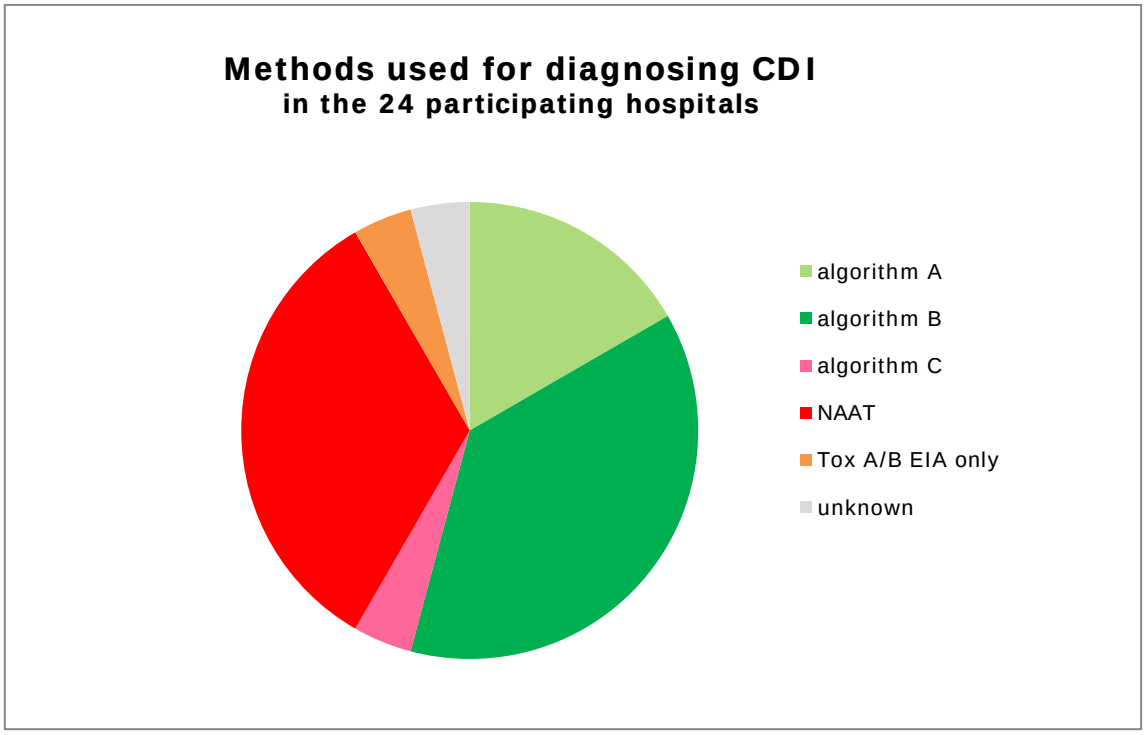


Figure 2. Laboratory methods used for diagnosing CDI in the 24 hospitals participating in the sentinel surveillance program. Algorithm A and B are recommended methods, all the others are non-recommended methods.

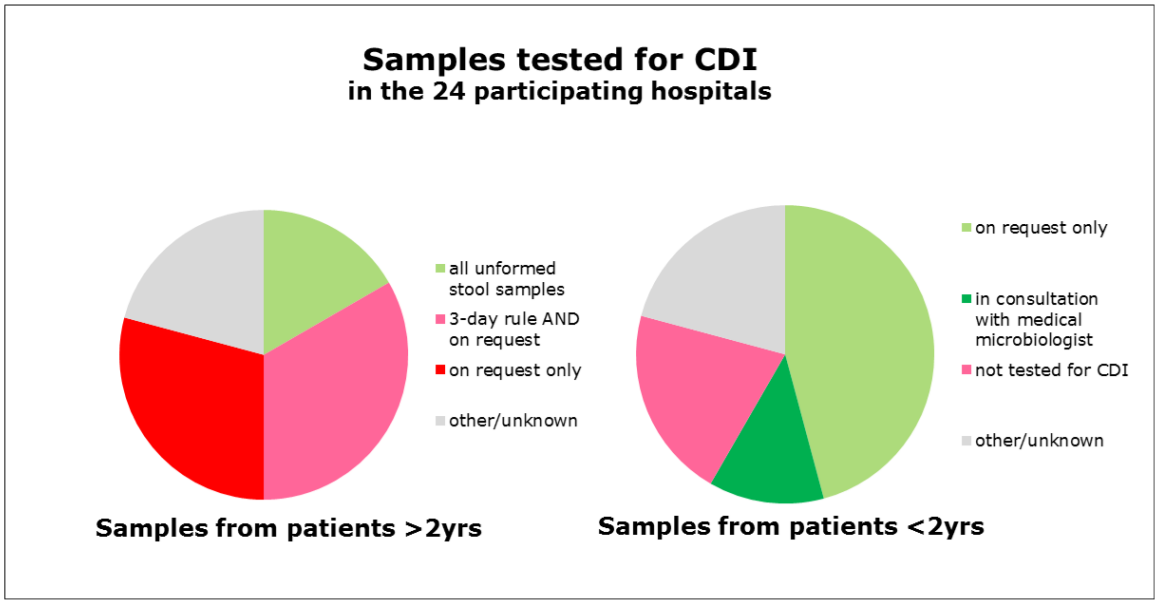


Figure 3. Samples tested for CDI in the 24 hospitals participating in the sentinel surveillance. Selection criteria for samples from patients >2 years are shown on the left, selection criteria for samples from patients <2 years are shown on the right.

Incidence in participating hospitals

The numbers of CDI per 10.000 patient-days per hospital are shown in Table 3, and compared to the incidence rate of the preceding year. The mean incidence was 3.08 CDI per 10.000 patient-days (varying from 0.68 to 6.62 CDI per 10.000 patient-days), comparable to the incidence of 3.09 that was reported in 2015-2016.[20] 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). In Figure 5, hospitals are split up into 3 groups depending on their selection criteria for CDI testing. For these 3 groups, incidence rates are shown.

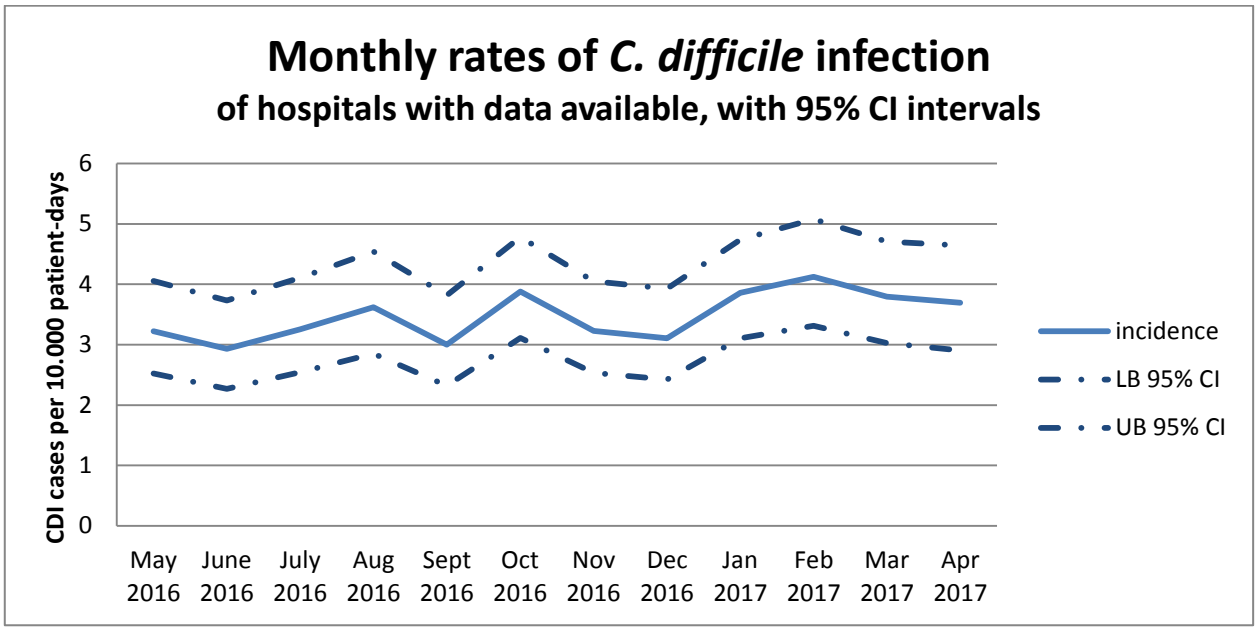


Figure 4. Monthly rates of *C. difficile* infection (cases per 10,000 patient-days) in 19 of the participating hospitals. LB 95% CI; lower bound 95% confidence interval, UB 95% CI; upper bound 95% confidence interval.

Submitted strains for PCR ribotyping

Of 1025 CDI patients included in sentinel surveillance between May 1st 2016 and May 1st 2017, 865 *C. difficile* isolates could be PCR ribotyped and linked to the clinical data (84%). The most important reasons for missing data were the inability to culture *C. difficile* at the local laboratory (n=69) or the inability to type *C. difficile* at the National Reference laboratory (culture negative or negative for GluD PCR, n=66).

Circulating PCR ribotypes

Similar as the previous year, ribotype 014/020 and 078/126 were the first and second most frequently isolated ribotypes. Ribotype 014/020 (indistinguishable by ribotyping) was isolated in 169 of the 865 samples (19.5%, 95% CI 16.9-22.2). The closely related ribotypes 078 and 126 were found in 105 samples (12.1%; 95% CI 10.0-14.3), ribotype 001 in 88 isolates (10.2%; 95% CI 8.2-12.2), ribotype 002 in 56 isolates (6.5%; 95% CI 4.8-8.1) and ribotype 005 in 43 isolates (5.0%; 95% CI 3.5-6.4). Five isolates were identified as ribotype 027 (0.6%; 95% CI 0.1-1.1) Of 51 isolates (5.0%, 95% CI 4.3-7.5) the PCR ribotype pattern was not recognized in our database. 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.

Figure 5. CDI incidence per 10.000 patient-days. Hospitals are stratified for their method of sample selection (testing of all unformed stool samples, 3-day rule and on request or on request only). Hollow circles represent hospitals using NAAT as a stand-alone test, solid circles represent hospitals using a recommended algorithm. Blue circles represent general hospitals, orange circles represent university-affiliated hospitals. The size of the circles reflects the hospital's yearly admission days.

Changes in circulating PCR ribotypes

The proportion of ribotype 001 increased compared to the previous years (2015-2016 95% CI 2.1- 4.7), this was mainly due to an outbreak caused by ribotype 001 in one of the participating hospitals (see below). The proportion of ribotype 027 was slightly lower than last year (2015-2016 95% CI 0.4-1.9) and significantly 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, 2014-2015 95% CI 0.1-1.3, see Figure 6). Ribotype 027 was found in five individual cases in 5 hospitals (5/24; 20.8%).

(Suspected) outbreaks in participating hospitals

In one of the participating hospitals located in the Eastern part of the Netherlands, an outbreak due to ribotype 001 was encountered. The outbreak started in January 2017 and coincided with a vancomycin resistant enterococci (VRE) outbreak on the same wards. From January 2017 till May 1st 2017, 33 ribotype 001 cases were reported to the reference laboratory.

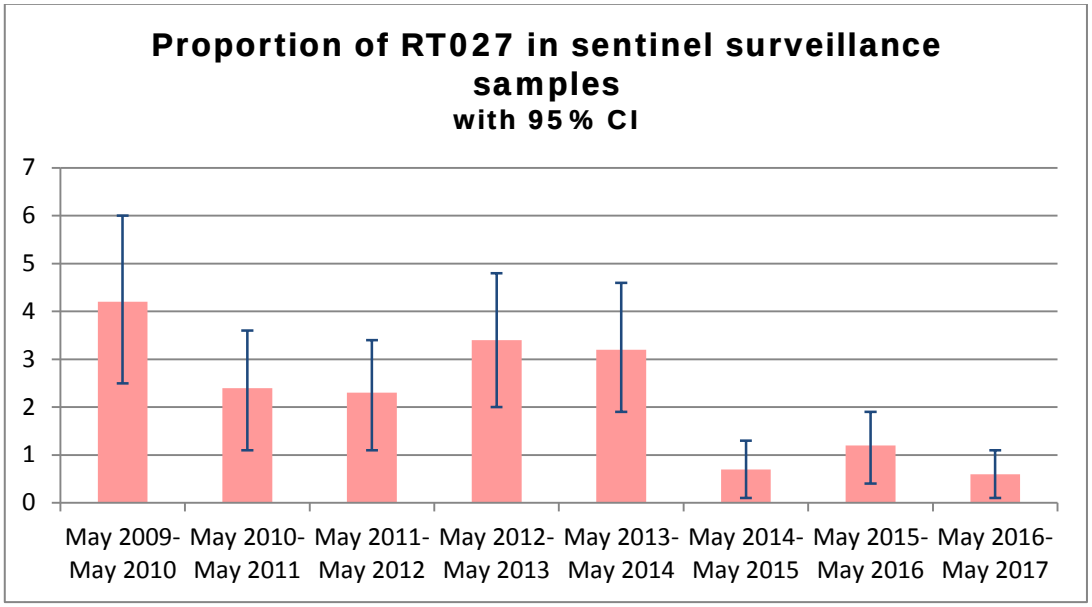


Figure 6. Proportion of 027 in sentinel surveillance samples. CI; confidence interval.

Demographical and clinical data

Demographical and clinical characteristics were collected from 1025 patients included in the sentinel surveillance (Table 1). The mean age was 66 years (95% CI 65.6-67.9). Of all patients, 2.7% (n=28) was younger than eighteen years old and 62.8% (n=644) was older than 65 years old. A total of 174 patients (17.4%) had severe CDI, defined as bloody diarrhoea and/or diarrhoea with hypovolemia or hypoalbuminemia (<20g/L) and/or with fever (T >38.0 °C) and leucocytosis (WBC count >15x10^9/l), and/or with pseudomembranous colitis. After 30 days, the outcome and course of the disease was known for 899 patients. In total 785 patients (87.3%) had an uncomplicated course of their CDI infection. On the other hand, 11 patients (1.2%) were admitted to the ICU as a consequence of CDI within 30 days, and 102 patients with CDI (11.3%) died. Nineteen deaths (2.1%) 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 community-onset cases, proportion of patients with severe CDI and proportion of patients with a complicated course of CDI were comparable.

Table 1. Clinical characteristics and outcome of patients (n=1025)

Patient characteristics and outcome	n / n[a]	%
Gender female	532/1022	52.0%
Location of onset CDI		
hospital	540/1022	52.8%
at home	420/1023	41.1%
nursing home	31/1023	3.0%
other health-care facility	32/1023	3.1%
Hospital department		
Internal Medicine	247/539	45,8%
Surgery	53/539	10,6%
Lung diseases and TB	47/539	8,7%
Geriatrics	28/539	5,2%
Gastroenterology	36/539	6,7%
Cardiology	27/539	5,0%
ICU	26/539	4,8%
Neurology	10/539	1,9%
Pediatrics	5/539	0,9%
Other or unknown	52/481	10,4%
Antibiotics prior to CDI	690/931	74.1%
Recurrence	189/702	26.9%
Severe diarrhoea	174/1002	17,4%
Pseudomembranous colitis	33/174	19,0%
Hypovolemia or hypo-albuminaemia	86/174	49,4%
Bloody diarrhoea	39/174	22,4%
Fever and leucocytosis	71/174	40,8%
Outcome		
Uncomplicated	785/899	87.3%
Surgery needed	1/899	0.1%
ICU admission needed	11/899	1.2%
Death, contributable to CDI	19/899	2.1%
Death, unrelated to CDI	81/899	9.0%
Death, cause unknown	2/899	0.2%

Table 2. Data from the sentinel surveillance for the period May 2016-May 2017 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
Incidence								
per 10.000 patient-days	2,7	2,8	2,9	2,9	2,9	3,0	3,1	3,1
Location of onset								
within heatlhcare facility	63%	73%	69%	63%	64%	59%	58%	59%
at home	37%	27%	31%	37%	36%	41%	42%	41%
Course and outcome								
Severe CDI	28%	20%	27%	25%	21%	24%	21%	17%
Uncomplicated course	66%	86%	87%	88%	87%	86%	89%	87%
Deaths contributable to CDI	4%	3%	4%	2%	3%	4%	2%	2%
PCR ribotype 027								
Prevalence	4.2%	2.4%	2.3%	3.4%	3.2%	0.7%	1.2%	0.6%
N reported 027 outbreaks-sentinel surveilance	1	1	0	1	0	0	0	0
N reported 027 outbreaks-ad hoc typing	2	2	1	2	5	1	0	1

Table 3. Number of patients included in the sentinel surveillance per hospital, and incidence data. Period: May 1st 2016 – May 1st 2017. 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	Months of participation	Monthly PD	Incidence per 10.000 PD 2016-2017	Incidence per 10.000 PD 2015-2016	Incidence difference
A	algorithm A	3-day rule AND on request	4.1% (16/392)	12	6114	0,68	1,51	-0,83
B	algorithm B	all unformed stool samples	3.9% (20/509)	12	3444	0,73	0,69	0,03
C	algorithm B	on request only	3.3% (34/1020)	12	15840	1,00	2,71	-1,71
D	algorithm B	all unformed stool samples	3.1% (42/1359)	12	5859	1,28	2,03	-0,75
E	NAAT*	3-day rule AND on request	9.9% (59/595)	12	9504	1,75	2,09	-0,34
F	algorithm A	3-day rule AND on request	3.2% (62/1931)	12	12000	1,87	2,48	-0,60
G	NAAT	other criteria[1]	6.5% (134/2076)	12	23408	1,99	2,52	-0,53
H	algorithm B	3-day rule AND on request	8.9% (26/292)	12	5537	2,11	NA	NA
I	algorithm B	other criteria[2]	6.1% (265/4348)	12	2887	2,31	5,88	-3,57
J	algorithm C	other criteria[3]	6.4% (142/2234)	12	11580	2,52	2,46	0,06
K	NAAT*	on request only	6.9% (248/3576)	12	13656	2,75	1,41	1,34
L	Tox A/B EIA	all unformed stool samples	5.4% (69/1279)	12	8399	2,88	3,77	-0,89
M	algorithm B	other criteria[2]	6.1% (265/4348)	12	15688	3,24	2,47	0,77
N	algorithm A	3-day rule AND on request	6.5% (104/1592)	12	15268	3,38	4,59	-1,21
O	NAAT*	on request only	8.5% (43/505)	12	3815	3,71	2,36	1,35
P	NAAT*	on request only	8.9% (87/975)	12	12325	3,99	3,57	0,42
Q	algorithm B	on request only	10.9% (112/1032)	12	13777	3,93	3,31	0,62
R	algorithm B	3-day rule AND on request	7.9% (122/1541)	12	12349	4,25	4,45	-0,19
S	NAAT*	3-day rule AND on request	7.2% (120/1661)	12	11151	4,41	2,99	1,42
T	NAAT*	on request only	8.6% (168/1943)	12	19941	4,43	3,20	1,23
U	NAAT*	3-day rule AND on request	7.3% (101/1381)	12	10354	4,51	4,80	-0,29
V	NA	NA	NA	12	6940	4,56	5,16	-0,60
W	algorithm B	all unformed stool samples	10.0% (101/1007)	12	8510	4,90	3,14	1,76
X	algorithm A	on request only	13.8% (182/1316)	12	14975	6,62	3,43	3,19
			7.1%	**144**		**3,08**	**3,09**	**-0,01**

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 or toxigenic culture) *(ESCMID recommended)*
Algorithm C: Tox A/B EIA – GDH EIA
*and culture of positive samples
[1]all unformed stool samples if stool sample was ordered manually, on request only if stool sample was ordered electronically, formed stool samples only tested in case of ICU admission and request
[2]all unformed stool samples, except patients from gastro-intestinal departments (then only if request for CDI)
[3]all unformed stool samples from inpatients, samples from outpatients if CDI test is requested

Table 4. The two most frequently found ribotypes per hospital, isolated amongst patients that were included in the sentinel surveillance. Period: 1st 2016 – May 1st 2017. Ribotype 014/020 are indistinguishable by conventional ribotyping, and ribotype 078/126 can be hardly discriminated.

Hospital	Samples		Sample	*C. difficile* positive		Most common type			2nd most common type		
	N	%	type*	N	%		N	%		N	%
A	5	0,5%	Isolates	3	60%	014/020	2	67%	005	1	33%
B	3	0,3%	Isolates	2	67%	005 and 046	both n=1	50% each	-		
C	19	1,9%	Isolates	17	89%	012, 054 and 078/126	all n=3	18% each	002	2	12%
D	9	0,9%	Isolates	8	89%	several[1]	all n=1	13% each	-		0%
E	20	2,0%	Isolates	12	60%	014/020	4	33%	several[2]	all n=1	8% each
F	27	2,6%	Isolates	26	96%	014 and 078/126	both n=4	15% each	005 and 023	both n=2	8% each
G	56	5,5%	Faeces	48	86%	078/126	10	21%	001	8	17%
H	14	1,4%	Faeces	13	93%	014/020 and 078/126	both n=4	31% each	several[6]	all n=1	8% each
I	8	0,8%	Isolates	8	100%	014/020	2	25%	several[7]	all n=1	13% each
J	35	3,4%	Isolates	34	97%	014/020	10	29%	several[3]	all n=2	6% each
K	45	4,4%	Isolates	45	100%	014/020	10	22%	078/126	7	16%
L	29	2,8%	Faeces	21	72%	078/126	6	29%	014/020	5	24%
M	61	6,0%	Isolates	47	77%	014/020	13	28%	078/126	6	13%
N	62	6,0%	Isolates	56	90%	014/020	9	16%	several[5]	all n=3	5% each
O	17	1,7%	Isolates	14	82%	014/020	4	29%	005 and 012	both n=2	14% each
P	59	5,8%	Isolates	37	63%	014/020	8	22%	078/126	7	19%
Q	65	6,3%	Isolates	65	100%	078/126	9	14%	014/020	6	9%
R	63	6,1%	Faeces	52	83%	014/020	12	23%	002	8	15%
S	59	5,8%	Isolates	49	83%	014/020	13	27%	078/126	8	16%
T	106	10,3%	Isolates	78	74%	014/020	12	15%	002 and 078/126	both n=9	both 12%
U	56	5,5%	Isolates	51	91%	014/020	6	12%	012 and 078/126	both n=5	10% each
V	38	3,7%	Isolates	37	97%	014/020	8	22%	078/126	4	11%
W	50	4,9%	Faeces	45	90%	014/020	10	22%	002 and 015	both n=6	13% each
X	119	11,6%	Faeces	97	82%	001	43	44%	014/020	19	20%
Total	**1025**	**100%**		**865**	**84%**	**014/020**	**169**	**19,5%**	**078/126**	**105**	**12,1%**

*Dominant sample type send to LUMC; **Number of patients of whom a ribotyping result could be linked to the clinical data in OSIRIS.
[1]001, 014, 057, 078/126, 081, 087
[2]003, 013, 021, 024, 029 and 078/126
[3]002, 015, 023, 052, 070 and 078/126
[4]001, 002, 015 and 175
[5]001, 002, 015 and 029
[6]002, 005, 011, 044, 059 and 137

Results of the ad hoc typing

Healthcare facilities and laboratories using the Reference Laboratory

In the period between May 1st 2016 and May 1st 2017, 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, 61 samples were submitted for ad hoc PCR ribotyping.

Ad hoc ribotyping results

C. difficile could be cultured from 87% of the 61 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 (17%). Other frequently found ribotypes were 014/020 (13%), 078/126 (11%), 001 (6%) and 002 (6%). The percentage of ribotype 027 decreased compared to last year, but varies in time: 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 increased compared to last year (4% in 2015-2016). 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).

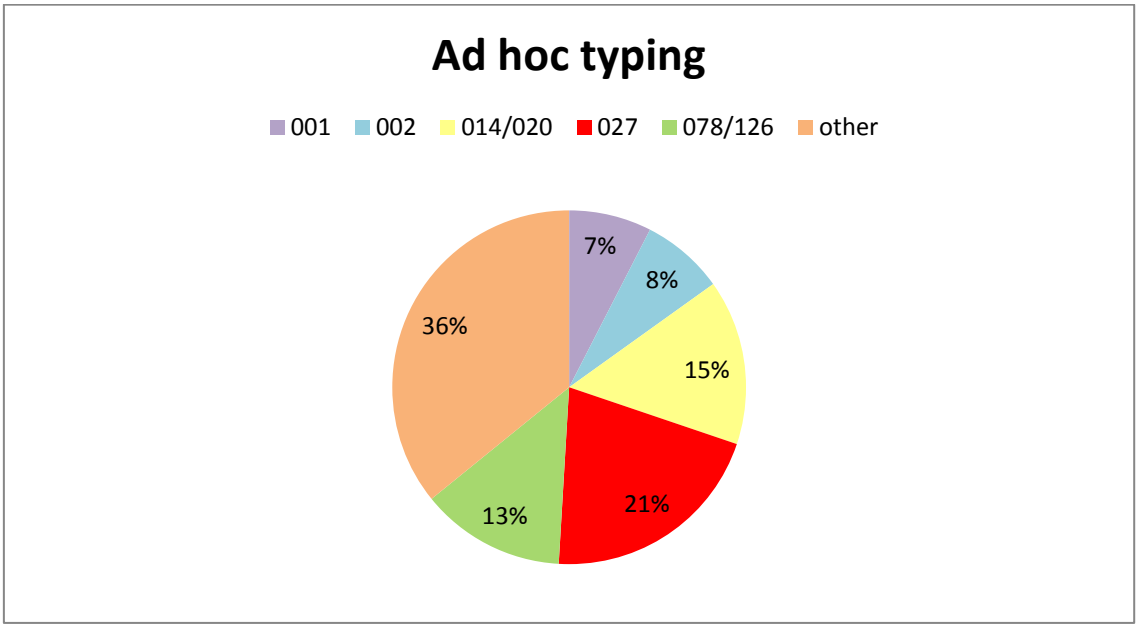
Outbreak investigation

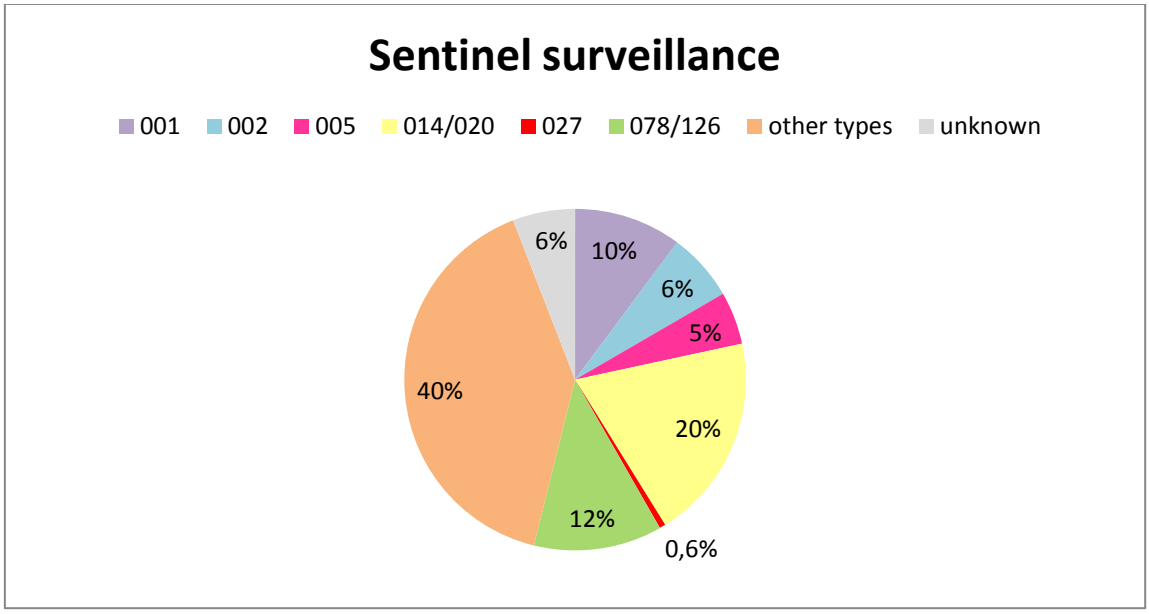
This year, one 027 outbreak was reported to the National Reference Laboratory. The outbreak took place at two different locations of one hospital in the western part of the Netherlands and comprised 9 patients within a 5-month period. By MLVA, isolates were genetically related to each other and to isolates from a previous outbreak in the same region which took place in 2015. CDI due to 027 was found in 1 other healthcare facility in the western part of the Netherlands; this patient had been admitted to the outbreak hospital within the 3 months before developing CDI. One of the individual 027 cases reported in one of the surveillance hospitals had also been admitted to this outbreak hospital and transmission was confirmed by MLVA.

Table 5. Results of the ad hoc typing. Period: May 1st 2016 – May 1st 2017. 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 ribotypes		
	N	%	type	N	%		N	%
1	3	5%	isolates	3	100%	001, 014, 078	all n=1	all 33%
2	17	28%	isolates	17	100%	014	5	29%
3	1	2%	feces	0	0%	-	-	-
4	1	2%	feces	1	100%	078	1	100%
5	1	2%	feces	0	0%	-	-	-
6	2	3%	feces	2	100%	001	2	100%
7	19	31%	isolates/feces	18	95%	027	10	56%
8	3	5%	feces	2	67%	070	2	100%
9	2	3%	feces	0	0%	-	-	-
10	8	13%	feces	6	75%	014, 015, 023, 106, 126, 293	all n=1	all 17%
11	1	2%	isolates	1	100%	010	1	100%
12	3	5%	isolates	3	100%	002, 027, 078	all n=1	all 33%
Total	**61**			**53**	**87%**	**027**	**11**	**17%**

Figure 7. 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 2016 – May 1st 2017. The category 'other types' consists of 79 different types in the sentinel surveillance data and 16 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 24 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 249 different PCR ribotypes.

Results of the sentinel surveillance (May 2016- May 2017)

- Diverse CDI diagnostic methods are applied, and just half of hospitals participating in the sentinel surveillance use optimal diagnostic methods as recommended by ESCMID and ECDC. Although recommended, most (83%) of the hospitals do not test all submitted unformed stool samples for CDI.
- A mean incidence rate of 3.08 CDI cases per 10.000 patient-days was found through sentinel surveillance (varying between hospitals from to 0.68 to 6.62 CDI cases per 10.000 patient-days), similar to last years.
- The disease severity was reported for 1002 out of 1025 patients included in the surveillance; 17% had severe CDI. The 30-day outcome was reported for 899 patients; 87% had un uncomplicated course, 1.2% was admitted to the ICU due to CDI. One of the patients needed surgery because of CDI. Outcomes of CDI were comparable to last year.
- 11% of the patients died within 30 days (n=102), for 19 patients (2.1%) their death was known to be contributable to CDI.
- One large outbreak due to ribotype 001 was reported in one the participating hospitals
- Similar as in 2015-2016, the most frequent encountered PCR ribotypes were ribotype 014/020 (20%) and the closely related ribotypes 078 and 126 (12%).
- Ribotype 027 was found in 0.6% of samples (1.2% during May 2015-May 2016)

Results of ad hoc typing (May 2016- May 2017)

- Twelve healthcare facilities/laboratories sent 61 samples to the Reference Laboratory for ad hoc typing because of outbreaks, severe CDI cases, or for other reasons.
- Ribotype 027 was the predominant ribotype (17%), followed by ribotypes 014/020 (13%) and ribotypes 078/126 (11%).
- An outbreak due to ribotype 027 (9 patients) was encountered in 1 healthcare facility, and 2 cases related to this outbreak were reported in other hospitals

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[26]), it is estimated that approximately 2895 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.

Output of the National Reference Laboratory May 2016-May 2017.

Publications related to the reference laboratory.

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).

Crobach MJ, Planche T, Eckert C, Barbut F, Terveer EM, Dekkers OM, Wilcox MH, Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for *Clostridium difficile* infection. Clin Microbiol Infect. 2016;22 Suppl 4:S63-81.

Kola A, Wiuff C, Akerlund T, van Benthem BH, Coignard B, Lyytikäinen O, Weitzel-Kage D, Suetens C, Wilcox MH, Kuijper EJ, Gastmeier P; members of ECDIS-Net. Survey of *Clostridium difficile* infection surveillance systems in Europe, 2011. Euro Surveill. 2016;21(29).

van Dorp SM, Notermans DW, Alblas J, Gastmeier P, Mentula S, Nagy E, Spigaglia P, Ivanova K, Fitzpatrick F, Barbut F, Morris T, Wilcox MH, Kinross P, Suetens C, Kuijper EJ; European Clostridium difficile Infection Surveillance Network (ECDIS-Net) project on behalf of all participants. Survey of diagnostic and typing capacity for *Clostridium difficile* infection in Europe, 2011 and 2014. Euro Surveill. 2016;21(29).

van Dorp SM, Kinross P, Gastmeier P, Behnke M, Kola A, Delmée M, Pavelkovich A, Mentula S, Barbut F, Hajdu A, Ingebretsen A, Pituch H, Macovei IS, Jovanović M, Wiuff C, Schmid D, Olsen KE, Wilcox MH, Suetens C, Kuijper EJ; European *Clostridium difficile* Infection Surveillance Network (ECDIS-Net) on behalf of all participants. Standardised surveillance of Clostridium difficile infection in European acute care hospitals: a pilot study, 2013. Euro Surveill. 2016:21(29).

Krutova M, Matejkova J, Kuijper EJ, Drevinek P, Nyc O; Czech *Clostridium difficile* study group. *Clostridium difficile* PCR ribotypes 001 and 176 - the common denominator of C. difficile infection epidemiology in the Czech Republic, 2014. Euro Surveill. 2016;21(29).

Crobach MJ, Terveer EM, Kuijper EJ. Effect of Detecting and Isolating Asymptomatic *Clostridium difficile* Carriers. JAMA Intern Med. 2016;176:1572-1573.

Nyc O, Tejkalova R, Kriz Z, Ruzicka F, Kubicek L, Matejkova J, Kuijper E, Krutova M. Two Clusters of Fluoroquinolone and Clindamycin-Resistant *Clostridium difficile* PCR Ribotype 001 Strain Recognized by Capillary Electrophoresis Ribotyping and Multilocus Variable Tandem Repeat Analysis.Microb Drug Resist. 2016 Nov 18. doi: 10.1089/mdr.2016.0159.

Krutova M, Nyc O, Matejkova J, Allerberger F, Wilcox MH, Kuijper EJ. Molecular characterisation of Czech *Clostridium difficile* isolates collected in 2013-2015. Int J Med Microbiol. 2016;306:479-485.

Terveer EM, Crobach MJ, Sanders IM, Vos MC, Verduin CM, Kuijper EJ. Detection of *Clostridium difficile* in Feces of Asymptomatic Patients Admitted to the Hospital. Clin Microbiol. 2017;55:403-41.

Orden C, Blanco JL, Álvarez-Pérez S, Garcia-Sancho M, Rodriguez-Franco F, Sainz A, Villaescusa A, Harmanus C, Kuijper E, Garcia ME. Isolation of *Clostridium difficile* from dogs with digestive disorders, including stable metronidazole-resistant strains. Anaerobe. 2017;43:78-81.
Andrés-Lasheras S, Bolea R, Mainar-Jaime RC, Kuijper E, Sevilla E, Martín-Burriel I, Chirino-Trejo M. Presence of *Clostridium difficile* in pig faecal samples and wild animal species associated with pig farms. J Appl Microbiol. 2017;122:462-472.

van Dorp SM, Smajlović E, Knetsch CW, Notermans DW, de Greeff SC, Kuijper EJ. Clinical and Microbiological Characteristics of *Clostridium difficile* Infection Among Hospitalized Children in the Netherlands. Clin Infect Dis. 2017;64:192-198.

Cairns MD, Preston MD, Hall CL, Gerding DN, Hawkey PM, Kato H, Kim H, Kuijper EJ, Lawley TD, Pituch H, Reid S, Kullin B, Riley TV, Solomon K, Tsai PJ, Weese JS, Stabler RA, Wren BW. Comparative Genome Analysis and Global Phylogeny of the Toxin Variant *Clostridium difficile* PCR Ribotype 017 Reveals the Evolution of Two Independent Sublineages. J Clin Microbiol. 2017;55:865-876.

Álvarez-Pérez S, Blanco JL, Harmanus C, Kuijper E, García ME. Subtyping and antimicrobial susceptibility of *Clostridium difficile* PCR ribotype 078/126 isolates of human and animal origin. Vet Microbiol. 2017;199:15-22.

Widmer AF, Frei R, Erb S, Stranden A, Kuijper EJ, Knetsch CW, Tschudin-Sutter S. Transmissibility of *Clostridium difficile* Without Contact Isolation: Results From a Prospective Observational Study With 451 Patients. Clin Infect Dis. 2017;64:393-400.

Aptekorz M, Szczegielniak A, Wiechuła B, Harmanus C, Kuijper E, Martirosian G. Occurrence of *Clostridium difficile* ribotype 027 in hospitals of Silesia, Poland. Anaerobe. 2017;45:106-113.

Beran V, Kuijper EJ, Harmanus C, Sanders IM, van Dorp SM, Knetsch CW, Janeckova J, Seidelova A, Barekova L, Tvrdik J, Chmelar D, Ciznar I. Molecular typing and antimicrobial susceptibility testing to six antimicrobials of *Clostridium difficile* isolates from three Czech hospitals in Eastern Bohemia in 2011-2012. Folia Microbiol (Praha). 2017. doi: 10.1007/s12223-01.

van Kleef E, Kuijper EJ, Bonten MJM, Cooper BS. *Clostridium difficile* in England: can we stop washing our hands? Lancet Infect Dis. 2017;17:478.

Krutova M, Nyc O, Matejkova J, Kuijper EJ, Jalava J, Mentula S. The recognition and characterisation of Finnish *Clostridium difficile* isolates resembling PCR-ribotype 027. J Microbiol Immunol Infect. 2017. pii: S1684-1182(17)30051-8.

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.

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

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

Presentations and posters at congresses.

NVMM, Annual meeting, Papendal, 11-13 April 2017.

E.M. Terveer, S.M. van Dorp, M. Fallon, A. Ormond, E. Everan, L. Kearns, M. Fitzpatrick, M.A. Caljouw, A.Martin, M. van Dorst, H.W. van Eijk, F. Fitzpatrick, E.J. Kuijper. Dynamic, low prevalence of *Clostridium difficile* and MDRO colonisation among nursing home residents in Ireland and the Netherlands.

I.G.J.M. Sanders, N. Kenters, E.G.W. Huijskens, S.C.J. de Wit, J. van Rosmalen, A. Voss, E.J. Kuijper. Effectiveness of various cleaning and disinfectant products on *Clostridium difficile* spores of PCR ribotypes 010, 014 and 027.

S.G. Hellmann, S.M. van Dorp, A.T.R. Tholen, C.M.P.M. Hertogh, E.J. Kuijper, M. Scholing. Prevalence and risk factors of *Clostridium difficile* colonization among residents of Long Term Care Facilities in Amsterdam, The Netherlands.

27th European Congress of Clinical Microbiology and Infectious Diseases. Vienna, 22-27 April 2017.

EP0361. Elisabeth Terveer, Yvette H. Van Beurden, Marcel Dijkgraaf, Els Van Nood, Abraham Goorhuis, M.P. Bauer, J.F.M. Seegers, Chris J.J. Mulder, Christina Vandenbroucke-Grauls, H.W. Verspaget, Josbert Keller, Ed J. Kuijper. Establishment and first experiences of the non-profit Netherlands Donor Faeces Bank.

EV0049. Sofie Maria Van Dorp, Emma Everan, Elisabeth Terveer, Laura Kearns, Margaret Fitzpatrick, Michael Alexander, Daisy Wopereis, Ingrid Verzijl, Monique Caljouw, Alan Martin, Ed J. Kuijper, Fidelma Fitzpatrick. Point-prevalence survey of *Clostridium difficile* and multidrug-resistant organism colonization among nursing-home residents in Ireland and the Netherlands.

EP0182. Stephan Hellmann, Sofie Maria Van Dorp, Aletta T.R. Tholen, Cees Hertogh, Ed J. Kuijper, Maarten Scholing. Prevalence and risk factors of *Clostridium difficile* colonization among residents of Long-term care facilities in Amsterdam, the Netherlands.

EP0183. Andreas F. Widmer, Reno Frei, Ed J. Kuijper, Mark H. Wilcox, Ruth Schindler, Violeta Spaniol, Daniel Goldenberger, Adrian Egli, Sarah Tschudin Sutter. *Clostridium difficile* point-prevalence study, Switzerland.

EP0185. Monique Jacqueline Theresia Crobach, Elisabeth Terveer, Kees Verduin, Margreet Vos, Ed J. Kuijper. Asymptomatic *Clostridium difficile* colonization on admission to a hospital: a multi-centre study.

P2029. Marcela Krutova, Otakar Nyc, Jana Matejkova, Ed J. Kuijper, Céline Harmanus, Arja Kanervo-Nordström, Jari Jalava, Silja Mentula. The recognition and characterization of Finnish *Clostridium difficile* PCR ribotype 027-like isolates.

P2042. Małgorzata Aptekorz, Anna Szczegielniak, Barbara Wiechuła, Céline Harmanus, Ed J. Kuijper, Gayane Martirosian. Faecal lactoferrin in patients infected with *Clostridium difficile* ribotype 027.

P0698. Elisabeth Terveer, Katarina Stein, Lynda Fenelon, Denise Drudy, Helen Lynch, Ingrid Sanders, Roel Nijhuis, Ed J. Kuijper. Prevalence of *Clostridium difficile* and colistin resistance gene (mcr-1) in faeces of piglets and sows in Ireland.

OS0223. Monique Jacqueline Theresia Crobach, Anne Voor in 't holt, Wilco Knetsch, Mark H. Wilcox, Sofie Maria Van Dorp, Margreet Vos, Ed J. Kuijper. A cluster of *Clostridium difficile* infections caused by a binary toxin-producing new PCR ribotype 826.

OS0225. Pete Kinross, Agnes Hajdu, Maja Rupnik, Jana Kolman, Virág Lesinszki, Judit Pászti, Aleksander Deptuła, Hanna Pituch, Ed J. Kuijper, Carl Suetens. EU-wide surveillance of *Clostridium difficile* provides opportunity to assess outcomes of *C. difficile* ribotype 027 infections.

P0486. Linda Verhoef, Ellen Stobberingh, Emma Smid, Ed J. Kuijper, Sabine De Greeff, Max Heck. Intestinal carriage of resistant bacteria and *Clostridium difficile* in nursing homes in the Netherlands - a point prevalence study.

ASM Microbe, Boston, 16-20 June 2016.

Ed J. Kuijper. Current Epidemiology of *C. difficile* Infection.

Anaerobe meeting, 11-14 July 2016, Nashville, TN.

EJ Kuijper, S. van Dorp, D Notermans, M. Crobach, de Greeff SC. Diagnosis and epidemiology of CDI in Europe.

ASMS, 5-9 June 2016, San Antonio, Texas.

Simone Nicolardi, Jeff Sen, Ingrid M.J.G. Sanders, Paul J. Hensbergen, Ed J. Kuijper. Recognition of *Clostridium difficile* PCR-Ribotypes by Intact Protein Analysis Using Ultrahigh Resolution MALDI-FTICR-MS.

Invited presentations.

Cursus Infectiepreventie, Oirschot. 19 January 2017. Ed J. Kuijper. *Clostridium difficile* infecties.

6Th AMIT (Topics in Infectious and Tropical Diseases) , Milan, 10 March 2017: Ed J. Kuijper. Diagnosis and treatment of CDI: state of the art.

Czech Society for Microbiology. 15 May 2017, Praque. Ed. J. Kuijper. CDI, a clinical diagnosis?

Karolinska Institute, Stockholm, 27 April 2017. EJ Kuijper, S. van Dorp, D Notermans, M. Crobach, de Greeff SC. *Clostridium difficile* infections; new data.

Participation and Organization of Workshops.

ECDC, Stockholm 14-15 September 2016. E CDC HAI-Net CDI surveillance.

Annual meeting of the Reference Laboratory, 28 October, 2017, RIVM.

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

References

(1) Smits WK, Lyras D, Lacy DB, Wilcox MH, Kuijper EJ. Clostridium difficile infection. Nat Rev Dis Primers. 2016 Apr 7;2:16020.

(2) Crobach MJT, Planche T, Eckert C et al. European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for Clostridium difficile infection. CMI 2016;22:S63-81

(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) Wilcox MH, Gerding DN, Poxton IR, Kelly C, Nathan R, Birch T, Cornely OA, Rahav G, Bouza E, Lee C, Jenkin G, Jensen W, Kim YS, Yoshida J, Gabryelski L, Pedley A, Eves K, Tipping R, Guris D, Kartsonis N, Dorr MB; MODIFY I and MODIFY II Investigators. Bezlotoxumab for Prevention of Recurrent Clostridium difficile Infection. N Engl J Med. 2017;376:305-317

(11) 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

(12) Terveer EM, van Beurden YH, Goorhuis A, Seegers JFML, Bauer MP, van Nood E, Dijkgraaf MGW, Mulder CJJ, Vandenbroucke-Grauls CMJE, Verspaget HW, Keller JJ, Kuijper EJ. How to: Establish and run a stool bank. Clin Microbiol Infect. 2017 May 18. pii: S1198-743X(17)30275-6).

(13) Steenbergen van J, Debast S, van Kregten E, 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.

(14) 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.

(15) 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.

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

(17) 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.

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

(19) 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.

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

(21) 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.

(22) 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.

(23) Jaarverslagen Zorg. www.jaarverslagenzorg.nl

(24) 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.

(25) 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.

(26) Dutch hospital data. Available at http://www.dutchhospitaldata.nl/