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

May 2013 - May 2014

Leiden University Medical Center, Department of Medical Microbiology

Drs. S.M. van Dorp, MD, PhD candidate
Ing. C. Harmanus, technician
Ing. I.M. Sanders, technician
Dr. J. Corver, molecular biologist
Prof. dr. E.J. Kuijper, medical microbiologist

Center for Infectious Diseases Control (CIb), RIVM, Bilthoven

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

Acknowledgements

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

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

Content

Summary

The National Reference Laboratory for *C. difficile*

As of 2005, outbreaks with "hypervirulent" *Clostridium difficile* PCR ribotype 027 were recognized in the Netherlands. Soon after their recognition, the Center for Infectious Disease Control (CIb) of the National Institute for Public Health and the Environment (RIVM) started a typing service for *C. difficile* at the Leiden University Medical Center (LUMC). This "ad hoc typing" service was offered to all microbiological laboratories in the Netherlands, and medical microbiologists were requested to send *C. difficile* samples from patients with severe *C. difficile* infection (CDI) and from outbreaks to the Reference Laboratory. As of May 2009, a sentinel surveillance was added with continuous monitoring of CDI in approximately 20 hospitals, including collection a minimum of clinical and epidemiological data and ribotyping of *C. difficile* isolates at the LUMC. Surveillance resulted in recognition of new emerging *C. difficile* PCR ribotypes, such as ribotype 078, which was also increasingly found in other European countries. In England, the prevalence of ribotype 078 has increased from 2% to 5% from 2007-2008 to 2009-2010, occurring during a remarkable decrease of the prevalence of ribotype 027.[1] Ribotype 078 emerged to one of the three most frequently found ribotypes in Scotland between 2009-2013 (prevalence in 2013 of 6%).[2]
The Reference Laboratory is now able to recognize 174 PCR ribotypes. In the previous year, eleven new PCR ribotypes were added to the Reference Laboratory library.

Results of the sentinel surveillance

The results of the sentinel surveillance in the period May 2013-May 2014 in 20 hospitals showed that the mean incidence of CDI was 16.2 per 10.000 hospital admissions, varying from 4 to 30 per 10.000 admissions. No outbreaks were reported by surveillance hospitals. The most frequent encountered PCR ribotypes included ribotype 014/020 (n=147/1014; 14.5%), the closely related ribotypes 078 and 126 (n=136/1014; 13.4%), and ribotype 001 (n=84/1014; 8.3%). Compared to the previous years, the proportion of ribotype 001 continued to decrease (2010-2011 20%, n=187, 2011-2012 17%, n=138, 2012-2013 14%, n=131). The proportion of ribotype 002 (n=73/1014; 7.2%) was slightly higher than last year (n= 50; 6%). Proportions of ribotype 005, ribotype 014/020, ribotype 027, and ribotypes 078/126 were similar to last year. No important new or emerging ribotypes were observed.
For 693 out of 811 patients included in the surveillance the disease severity was reported; 21% had severe CDI. After 30 days, the outcome was reported for 635 patients; 2% of the patients (n=15) were admitted to the ICU and 11% died (n=70), of which 18 patients known to be contributable to CDI.

Results of ad hoc typing

In the period between May 1st 2013 and May 1st 2014, 18 facilities sent n=174 strains to the Reference Laboratory for ad hoc typing, because of outbreaks or severe cases of CDI. Ribotype 027 was the predominant ribotype, found in 31.7%, followed by ribotype 078/126 (11.2%). Seven outbreaks were reported; five of these outbreaks were caused by ribotype 027 and two by ribotype 001. Two hospitals needed extensive infection control measures, including antibiotic stewardship with restriction of fluoroquinolone use, to control 027 outbreaks. The 027 outbreak strain from one of these hospitals seems to have transmitted to at least seven other hospitals and seven nursing homes, using MLVA.

Burden of CDI in the Netherlands

Extrapolating the data of sentinel surveillance to all hospitals in the Netherlands, it is estimated that more than 3000 hospitalized patients annually will develop CDI. We estimated that 120 patients succumb contributable to CDI annually. In these estimations, the impact of CDI in other healthcare facilities than hospitals was not included.
By "ad hoc typing" it was observed that ribotype 027 re-emerged in several Dutch healthcare facilities and caused five outbreaks in the last year. Since the Reference Laboratory confirmed ninety cases and another seventy cases were typed locally, we estimate a burden of at least 160 patients affected by ribotype 027 in May 2013- May 2014. MLVA showed transmission of an 027 subtype between several healthcare facilities, in different regions of the Netherlands. Therefore, alertness for a re-emergence of ribotype 027 is warranted.

[1] Wilcow et al. Changing epidemiology of *Clostridium difficile* infection following the introduction of a national ribotyping-based surveillance scheme in England. Clin Infect Dis. (2012) 55 (8): 1056-1063. doi: 10.1093/cid/cis614
[2] Wiuff et al. Changing epidemiology of *Clostridium difficile* ribotypes in Scotland between 2009-2013 O005, ECCMID Barcelona 2014.

Introduction

Clostridium difficile is an anaerobic bacterium that is capable of producing toxins which are associated with diarrhoea. *C. difficile* can be divided in more than 500 PCR ribotypes and 24 toxinotypes. PCR ribotyping is based on differences in profiles generated by PCR amplification of the intergenic spacer regions between the 23S and 16S rRNA genes. At the Reference Laboratory in Leiden, currently, 174 different types have been included in the database. Toxinotyping involves detection of polymorphisms in the toxin A and B and surrounding regulatory genes, an area of the genome known collectively as the pathogenicity locus or PaLoc.[3]

Clostridium difficile infection (CDI) varies from mild diarrhoea to severe colitis or a life-threatening pseudomembranous colitis. Since 2003, an increasing incidence of CDI worldwide has been noticed. A new hypervirulent strain of *C. difficile* (PCR ribotype 027, North American pulsed-field type 1 (NAP1), restriction endonuclease analysis (REA) group BI) was (partially) the cause of this changed epidemiology. CDI due to riboype 027 is associated with a higher morbidity and mortality and has tendency to relapse more frequently.

As of 2005, outbreaks with ribotype 027 were also recognized in the Netherlands. Soon after their recognition, the Center 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, available for all microbiology laboratories in the Netherlands. All medical microbiologists in the Netherlands were requested to send *C. difficile* samples from patients with severe CDI and from outbreaks to the Reference Laboratory. These samples were cultured and, when identified as *C. difficile,* subtyped into one of the 174 PCR ribotypes that are known by the Reference Laboratory. Together with the submission of samples (faeces samples or *C. difficile* isolates), laboratories were requested to submit a standardized clinical questionnaire. Additional genetic typing and antibiotic susceptibility patterns were determined in special cases, such as outbreak strains or on request of the physician.

Data from the Reference Laboratory contributed to recognition of emerging types in the Netherlands with their clinical characteristics and the finding of type specific risk factors for CDI.[4 5 6] Furthermore, a decrease in ribotype 027 was noted, together with an increase of the more virulent ribotype 078.[7] This type has also been found as an increasing type in Europe and is currently the third most frequent found type European wide.[8] Interestingly, *C. difficile* ribotype 078 has also been recognized as the most important agents of piglet-associated diarrhoea[9], and humans and swine carry genetically identical strains based on whole-genome sequencing.[10 11]

In order to study the incidence of CDI in an endemic situation, a new sentinel surveillance started in May 2009. Approximately twenty hospitals participated in this surveillance and introduced a continuous monitoring of CDI. This is the eighth 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 2013 and May 1st 2014.

---

[3] Knetsch et al. Current application and future perspectives of molecular typing methods to study *Clostridium difficile* infections, Euro Surveill. 2013; Jan 24;18(4):20381.

[4] Goorhuis et al. Emergence of *Clostridium difficile* infection due to a new hypervirulent strain, PCR-ribotype 078, Clin. Infect. Dis. 2008;47:1162-70

[5] Goorhuis 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

[6] Goorhuis et al. Type-specific risk factors and outcome in an outbreak with 2 different *Clostridium difficile* types simultaneously in 1 hospital, Clin Infect Dis. 2011 Nov;53(9):860-9. doi: 10.1093/cid/cir549. Epub 2011 Sep 13.

[7] Hensgens et al. Decrease of hypervirulent *Clostridium difficile* PCR ribotype 027 in the Netherlands, Euro Surveill. 2009;14(45).pii=19402

[8] Bauer MP et al, ECDIS Study Group. *Clostridium difficile* infection in Europe: a hospital-based survey. Lancet. 2011;377:63-73.

[9] Debast SB et al. *Clostridium difficile* PCR ribotype 078 toxinotype V found in diarrhoeal pigs identical to isolates from affected humans. Environ Microbiol. 2009;11:505-11

[10] Knetsch CW. Whole genome sequencing revealed transmission of *Clostridium difficile* between humans and farm animals in the Netherlands. Oral presentation: NVMM, 15th of April 2014.

[11] Keessen EC et al. *Clostridium difficile* infection associated with pig farms. Emerg Infect Dis. 2013 Jun;19(6):1032-4. doi: 10.3201/eid1906.121645

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.

Ad 1. The hospitals participating in the sentinel surveillance are requested to include all hospitalized CDI patients >2 years old with a positive toxin test 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* send strains to the laboratory of the Leiden University Medical Center. Other laboratories send faecal samples. Isolation and characterization of *C. difficile* are performed as described in the next chapter. To calculate incidence rates, we requested the participating hospitals to register their number of admissions and number of admission-days. If these data were not submitted, they were extracted from the most recent annual report. Incidence rates are expressed in number of CDI patients divided by the number of hospital admissions and the number of admission-days, respectively. Actual incidence rates might be slightly underestimated, as children below 2 years old are excluded from the surveillance, but are included in the denominator data for feasibility.

Ad 2. All faecal samples are cultured and *C. difficile* isolates are characterized at the laboratory of the Leiden University Medical Center. Once a week, the results of the microbiological analysis are sent by e-mail to the submitting microbiologist, infection control practitioners, and to CIb. The results are also reported in Osiris. The Osiris system is used to complete online questionnaires of patients who are affected with CDI (see Ad 3). Besides these questionnaires, also the results of the PCR ribotyping are displayed on this site. When PCR ribotype 027 is found, the laboratories are also informed by telephone and are asked to contact the LUMC or CIb for additional information and advices. All submitting laboratories also receive an official report by regular post. Once a year, an overview of the results of the sentinel surveillance is provided to the participating hospitals.

Ad 3. The participating hospitals are asked to complete an online questionnaire of all patients included in the sentinel surveillance. This questionnaire is available at https://osiris.rivm.nl/cdif and contains questions involving patients sex, age, acquisition 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.[12] [13] In this online database OSIRIS, 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. Statistical analysis are performed using Excel and SPSS for Windows software package, version 20.

[12] Kuijper et al. Emergence of *Clostridium difficile*-associated disease in North America and Europe. Clin Microbiol Infect 2006; 12 Suppl 6:2-18

[13] McDonald et al. Recommendations for surveillance of *Clostridium difficile*-associated disease. Infect Control Hosp Epidemiol 2007; 28(2):140-5

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 the 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.

Ad 2. Isolation of *C. difficile* from faeces samples at the Reference laboratory is performed on *C. difficile* selective agar supplemented with cefoxitine, amphotericine 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 homemade PCR.

Ad 3. All isolates are genetically identified as *C. difficile* by an in-house PCR for the presence of the *glu*D gene, encoding the glutamate dehydrogenase (GDH) specific for *C. difficile*.[14] All *C. difficile* strains are further investigated by PCR-ribotyping.[15] The presence of *tcdA*, *tcdB* and binary toxin genes can be investigated according to standardized techniques on request.[16] [17] [18] Deletions in *tcd*C can be determined by PCR using in-house designed primers.[19]

Ad 4. 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 a telephone call and are offered to contact the LUMC or the CIb for additional information and advices. Submitting laboratories also receive an official report by regular post.

Ad 5. A standardized questionnaire is used to obtain information on patient's age and sex, the ward where CDI was acquired, clinical data, risk factors, antibiotic treatment in the month preceding a positive test and treatment outcomes. Co-morbidity is defined according to the ICD-10 classification. The questionnaires are sent by e-mail to the submitting laboratories when faecal samples are received. All analyses are performed using the SPSS for Windows software package, version 20. The definitions applied in this questionnaire are based on those proposed by the ECDC and the CDC.[20] [21]

[14] Paltansing et al. Characteristics and incidence of *Clostridium difficile*-associated disease in The Netherlands, 2005. Clin Microbiol Infect 2007;13:1058-64
[15] Bidet et al. Comparison of PCR-ribotyping, arbitrarily primed PCR, and pulsed-field gel electrophoresis for typing *Clostridium difficile*. J Clin Microbiol 2001;38:2484-7
[16] Goncalves et al. Prevalence and characterization of a binary toxin (actin-specific ADP-ribosyltransferase) from *Clostridium difficile*. J Clin Microbiol 2004;42:1933-9
[17] Kato et al. Identification of toxin A-negative, toxin B-positive *Clostridium difficile* by PCR. J Clin Microbiol 1998;36:2178-82
[18] Kato H et al. Deletions in the repeating sequences of the toxin A gene of toxin A-negative, toxin B-positive *Clostridium difficile* strains. FEMS Microbiol Lett 1999;175:197-203
[19] Kuijper et al. *Clostridium difficile* ribotype 027, toxinotype III, the Netherlands. Emerg Infect Dis 2006; 12:827-30
[20] Kuijper et al. Emergence of *Clostridium difficile*-associated disease in North America and Europe. Clin Microbiol Infect 2006; 12 Suppl 6:2-18
[21] McDonald et al. Recommendations for surveillance of *Clostridium difficile*-associated disease. Infect Control Hosp Epidemiol 2007;28:140-5

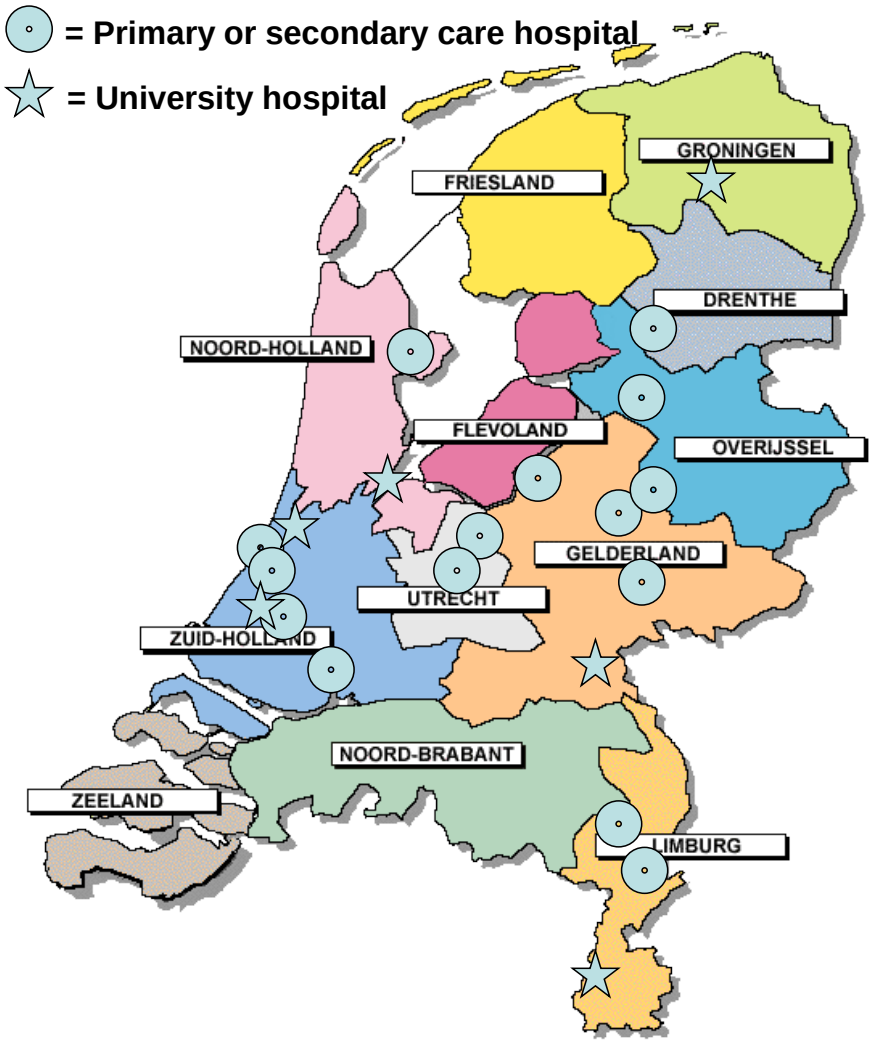
Results of the sentinel surveillance

Participating hospitals

This section describes the results of the current 21 participating hospitals. Both university hospitals (n=6) and primary or secondary care hospitals (n=15) were included, distributed all over the Netherlands. The geographical location of the participating centres is displayed in figure 1. Not all hospitals participated in the surveillance during the full twelve months (table 2). Two new participants (H and E) started in September 2013 and December 2013 respectively, and one hospital (N) interrupted surveillance from October 2012 to November 2013. Hospital K interrupted surveillance in March 2014. One hospital (U) started surveillance after a ribotype 027 outbreak in August 2013. To avoid any bias of national estimations, results of this hospital will be described separately.

The primary diagnostic tests used by the participating hospitals to diagnose CDI is depicted in (table 3). By May 2014, 24% (n=5) of the hospitals used an enzyme immunoassay for toxins, 33% (n=7) used an assay detecting both glutamate dehydrogenase and toxins, and 43% (n=9) used PCR or Loop-Mediated Isothermal Amplification. Between May 2013 and May 2014, five hospitals switched from using toxin enzyme immunoassays to more sensitive diagnostic tests, such as assays detecting glutamate dehydrogenase and LAMP.

Figure 1. Participating hospitals of the sentinel surveillance



Circulating PCR ribotypes

The Reference Laboratory received more samples from surveillance hospitals for PCR ribotyping, than of patients included in the sentinel surveillance. The additional samples were collected during time periods other than active surveillance, or arrived from non-hospitalized patients (outpatients, nursing home patients, or community patients). Between May 1st 2013 and May 1st 2014, the 21 participating hospital submitted 1188 samples, from which 1049 (88%) yielded *C. difficile.* Thirty-five samples were send by hospital U, that started surveillance because of an outbreak. These samples are excluded from the subsequent paragraph, as explained earlier.

Ribotype 014/020 (indistinguishable by ribotyping) was the most frequently found type, isolated in 147 of the 1014 isolates (14.5%, 95% CI 12.5-16.8). The closely related ribotypes 078/126 were found in 136 isolates (13.4%; 95% CI 11.5-15.6), ribotype 001 in 84 isolates (8.3%; 95% CI 6.7-10.1), ribotype 002 in 73 isolates (7.2%; 95% CI 5.8-9.0) and ribotype 005 in 54 isolates (5.3%; 95% CI 4.1-6.9). Thirty-four isolates were identified as ribotype 027 (3.4%; 95% CI 2.4-4.6) and one 027-like PCR ribotype (176)[22] was found. Of 88 isolates (8.7%, 95% CI 7.1-10.6) the PCR ribotype pattern was not recognized in our database, which is similar as last year (6.7%; 95% CI 5.2-8.5). The results stratified per participating centre are displayed in Table 2. A pie-chart of the five most common ribotypes of patients included in the sentinel surveillance is illustrated in figure 4.

Compared to the previous years, the proportion of ribotype 001 continued to decrease (2010-2011 n=187, 20%; 95% CI 17.7-22.9, 2011-2012 n=138, 17%; 95% CI 14.2-19.2, 2012-2013 n=131, 14%, 95% CI 12.3-16.8). The proportion of ribotype 002 (n=73/1014; 7.2%) was slightly higher than last year (2012-2013 n=50, 6%; 95% CI 4.2-7.2). Proportions of ribotype 005, ribotype 014/020, ribotype 027, and ribotypes 078/126 were similar to last year. No important new or emerging ribotypes were observed.

On a hospital level, the two predominant ribotypes were the same as last year within four hospitals (F,K,Q,S,T). One of the predominant ribotypes changed within seven hospitals (A,B,I,J, L,N,O), and both predominant ribotypes changed within six hospitals (C,D,G,M,P,R). Ribotype 027 was found in more than half of the hospitals (11/20; 55%), not taking into account the hospital that initiated surveillance because of an 027 outbreak.

Outbreaks in participating hospitals

Between May 1st 2013 and May 1st 2014, no outbreaks were observed in participating hospitals of the sentinel surveillance. 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. Hospital O did have a peak in the CDI incidence in November 2013 and a cluster of ribotype 001 (n=5), but three of these patients had their onset of symptoms in the community or in a nursing home, and therefore the cluster did not fulfil the outbreak definition.

Incidence in participating hospitals

The incidence rates of CDI were calculated over periods of active surveillance, and are shown in table 3. As mentioned before, data of the hospital that started surveillance because of an outbreak were excluded from national estimations, to avoid bias. Denominator data were provided by the hospital infection control personnel or microbiologist. If not available, denominator data were subtracted from hospital annual reports. The mean incidence was 16.2 per 10.000 hospital admissions (varying from 4.4 to 29.8 per 10.000 admissions), slightly higher than the incidence of 15 per 10.000 admissions that was reported in 2010-2013.[23]

[22] Knetsch et al. Comparative analysis of an expanded *Clostridium difficile* reference strain collection reveals genetic diversity and evolution through six lineages. Infect Genet Evol. 2012 Oct;12(7):1577-85. doi: 10.1016/j.meegid.2012.06.003. Epub 2012 Jun 15.

[23] The fifth, sixth and seventh annual report of the National Reference Laboratory for *Clostridium difficile* infection, available at http://www.rivm.nl/Onderwerpen/C/Clostridium/Clostridium_difficile

Demographical and clinical data

Demographical and clinical characteristics were collected from 811 patients included in the sentinel surveillance, hospital U included. Results are summarized in table 1. The mean age was 67 years (SD 19.0), varying from 3 to 101 years. Of all patients, 2.7% (n=22) was younger than eighteen years old. A total of 143 patients (21%) 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 leukocytosis (WBC count >15x10^9/l), and/or with pseudomembranous colitis. After 30 days, the outcome and course of the disease was known for 635 patients. After 30 days, 550 patients (87%) had an uncomplicated course of their CDI infection. On the other hand, 15 patients (2%) were admitted to the ICU as a consequence of CDI within 30 days, and 70 patients with CDI (11%) died. Eighteen deaths (3%) were contributable to CDI; four of these infections were caused by ribotype 001, two by ribotype 002, two by ribotype 014/020, and two by ribotype 078/126. The other infections were caused by ribotype 017, ribotype 045, ribotype 054, and an unknown ribotype. Of the remaining four patients, *C. difficile* could not be isolated and typed. No deaths solely due to CDI were reported this year, and none of the patients needed surgery as a consequence of their CDI.

Table 1. Clinical characteristics and outcome of patients

Patient characteristics and outcome	N	%
Gender female	416/811	51%
Location of onset CDI		
▪ Hospital	432/775	56%
▪ At home	281/775	36%
▪ Nursing home	33/775	4%
▪ Other health-care facility	29/775	4%
Hospital department		
▪ Internal Medicine	121/421	29%
▪ Surgery	53/421	13%
▪ Lung diseases and TB	34/421	8%
▪ Cardiology	28/421	7%
▪ Geriatrics	24/421	6%
▪ ICU	23/421	5%
▪ Gastroenterology	21/421	5%
▪ Neurology	20/421	5%
▪ Other	97/421	23%
Antibiotics prior to CDI	450/659	68%
Recurrence	117/495	24%
Severe diarrhoea	143/693	21%
▪ Pseudomembranous colitis	22/143	15%
▪ Hypovolemia or hypoalbuminemia	77/143	54%
▪ Bloody diarrhoea	29/143	20%
▪ Fever and leucocytosis	44/143	31%
Outcome		
▪ Uncomplicated	550/635	87%
▪ ICU admission needed	15/635	2%
▪ Death, contributable to CDI	18/635	3%
▪ Death, unrelated to CDI	43/635	7%
▪ Death, cause unknown	9/635	1%

Table 2. Isolates submitted for PCR ribotyping by hospitals participating in the sentinel surveillance. Period: May 1st 2012 – May 1st 2013. 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 U, which started surveillance because of an outbreak, is shown separately to avoid bias.

Hospital	Samples		Sample	C. difficile		Most common Type			2nd most common Type		
	N	%	type*	N	%		N	%		N	%
A	3	0,3%	Isolates	3	100%	078/126	2	67%	002	1	33%
B	10	0,9%	Isolates	9	90%	Unknown	2	22%	001	1	11%
C	16	1,4%	Isolates	16	100%	Unknown	3	19%	005	2	13%
D	17	1,5%	Isolates	17	100%	014/020	5	29%	002	3	18%
E	21	1,8%	Isolates	20	95%	014/020	6	30%	Unknown	4	20%
F	25	2,2%	Isolates	25	100%	001	7	28%	014/020	4	16%
G	46	4,0%	Faeces	24	52%	078/126	4	17%	Unknown	4	17%
H	154	13,4%	Isolates	125	81%	014/020	17	14%	078/126	15	12%
I	37	3,2%	Faeces	27	73%	014/020	5	19%	078/126	4	15%
J	88	7,6%	Faeces	65	74%	014/020	13	20%	005	8	12%
K	64	5,6%	Isolates	63	98%	078/126	16	25%	014/020	7	11%
L	46	4,0%	Faeces	37	80%	014/020	5	14%	015	5	14%
M	63	5,5%	Faeces	51	81%	078/126	9	18%	002	7	14%
N	106	9,2%	Isolates	105	99%	014/020	16	15%	078/126	14	13%
O	56	4,9%	Isolates	45	80%	001	20	44%	Unknown	5	11%
P	35	3,0%	Isolates	29	83%	014/020	7	24%	002	4	14%
Q	111	9,6%	Isolates	107	96%	014/020	13	12%	001	12	11%
R	108	9,4%	Isolates	103	95%	078/126	22	21%	014/020	12	12%
S	77	6,7%	Isolates	76	99%	078/126	11	14%	014/020	10	13%
T	70	6,1%	Isolates	67	96%	014/020	11	16%	027	6	9%
Total	**1153**	**100%**		**1014**	**88%**	**014/020**	**147**	**14,5%**	**078/126**	**136**	**13,4%**
U**	35	2,9%	Isolates	35	100%	014/020	5	14%	027	5	14%

*Dominant sample type send to LUMC

Table 3. Number of patients included in the sentinel surveillance per hospital, and incidence data. Period: May 1st 2013 – May 1st 2014. The incidence data are calculated using months of active participation. *Hospital U, which started surveillance because of an outbreak, is shown separately to avoid bias. The primer diagnostic test for CDI is shown per hospital; if the applied diagnostic test changed during the surveillance period, two subsequent tests are illustrated. The incidence per 10.000 admissions is compared the results of the previous annual report, demonstrated as an incidence difference.

Participating hospital	Months of participation	Patients N (%)		Monthly admissions	Monthly patient-days	Incidence per 10.000 admissions	Incidence per 10.000 patient-days	Diagnostic test	Incidence per 10.000 admissions 2012-2013	Incidence difference
A	12	4	0,5%	760	3753	4,4	0,9	EIA[1]	8,3	-3,9
B	12	6	0,8%	753	3726	6,6	1,3	EIA[2]; EIA[3]	11,7	-5,1
C	12	13	1,7%	1174	5942	9,2	1,8	EIA[3]	20,6	-11,4
D	12	17	2,2%	1297	6347	10,9	2,2	EIA[1]; EIA[4]	9,1	1,8
E	5	20	2,6%	2440	16835	16,4	2,4	EIA[3]	NA	NA
F	12	26	3,4%	1364	6301	15,9	3,4	LAMP	15,3	0,6
G	12	28	3,6%	1882	9360	12,4	2,5	EIA[2]	8,2	4,2
H	8	33	4,3%	2772	14599	14,9	2,8	PCR	NA	NA
I	12	36	4,6%	2608	13043	11,5	2,3	EIA[2]; EIA[3]	10,3	1,2
J	12	37	4,8%	1854	11655	16,6	2,6	EIA[1]	15,1	1,5
K	10	39	5,0%	2398	15359	16,3	2,5	PCR	23,7	-7,4
L	12	42	5,4%	2938	16880	11,9	2,1	PCR	16,2	-4,3
M	12	51	6,6%	3148	24254	13,5	1,8	EIA[1]; LAMP	5,5	8,0
N	6	52	6,7%	3058	25504	28,3	3,4	PCR	11,4	16,9
O	12	54	7,0%	2248	10677	20,0	4,2	EIA[2]; EIA[3]	15,8	4,2
P	12	56	7,2%	2217	13333	21,0	3,5	EIA[1]	19,3	1,7
Q	12	60	7,7%	2377	13386	21,0	3,7	EIA[1]	23,0	-2,0
R	12	64	8,2%	2194	11429	24,3	4,7	LAMP	27,0	-2,7
S	12	68	8,8%	2899	16938	19,5	3,3	EIA[3]	15,3	4,2
T	12	70	9,0%	1960	10158	29,8	5,7	PCR	19,1	10,7
Total		**776**	**100%**	**42341**	**249477**	**16,2**	**2,9**		**14,7**	**1,5**
U*	9	35	4,3%	1483	6982	26,2	5,6	LAMP	NA	NA

NA= not available

LAMP= Loop-Mediated Isothermal Amplification

EIA[1]= Toxin assay (Vidas®)

EIA[2]= Toxin assay (Immunocard®)

EIA[3]= GDH and toxin assay (C. diff Quik Chek Complete® or Liaison®)

Results of the ad hoc typing

Hospitals using the Reference Laboratory

In the period between May 1st 2013 and May 1st 2014, 18 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 surveillance, such as severe CDI or suspicion of an outbreak. Some of these facilities sent only a part of their outbreak strains for typing, therefore numbers of cases involved in outbreaks as reported may underestimate the total number of outbreak cases. Facility 3 started surveillance in August 2013 because of a ribotype 027 outbreak ('hospital U'), samples send before that time are described this chapter. In total, 174 samples were submitted for ad hoc PCR ribotyping.

Ad hoc ribotyping results

Of the 174 samples submitted, 93% contained *C. difficile.* This percentage is significantly higher than last year (83%). The number of submitted isolates and most common PCR ribotypes, stratified per facility, are demonstrated in table 4. Ribotype 027 was the most commonly found PCR ribotype (31.7%), followed by ribotype 078/126 (11.2%), ribotype 001 (9.3%) and 014/020 ribotype (indistinguishable by ribotyping, 9.3%). The percentage of ribotype 027 increased compared to last year, and fluctuates in time: 20% in 2012-2013, 15% in 2011-2012, 26% in 2010-2011, and 4% in 2009-2010. Between 2005 and 2007 the proportion of ribotype 027 was 12% in 2006-2007 and 21% in 2005-2006.

Outbreak investigation

Since 1st of May 2013, seven outbreaks were observed by the Reference Laboratory. An outbreak was defined as >2 isolates of the same type, found less than 7 days apart in one hospital. Data on hospital ward and other patient characteristics were not always available. Five outbreaks were caused by ribotype 027 and two by ribotype 001. The number of 027 outbreaks was remarkably higher than we observed in the prior years, when one or two 027 outbreaks a year were reported.

Two outbreaks of ribotype 001 occurred in facility 1 in May (n=4) and July 2013 (n=3). Ribotype 027 caused outbreaks in facility 3 in April-May (n=3) and in June 2013 (n=4). This facility experienced 027 outbreaks before, and again enhanced infection control (improvement of basic hygiene and temporal ban on quinolones) to control the outbreak. Subsequently, the facility started sentinel surveillance in August 2013. Ribotype 027 also caused a major outbreak in facility 8, starting in May 2013. The number of cases decreased in June 2013, but there were recurrent peaks in September and November 2013. The outbreak was controlled by strict isolation of all diarrheic patients (including non-CDI patients), and a restriction of ciprofloxacin use. In total, 79 cases occurred between start of the outbreak and April 2014. Facility 6 sent several 027 samples from nursing homes in the same region as facility 8, without having outbreaks. A fourth 027 outbreak (n=3) occurred in a nursing home (facility 10) in September 2013. The first case was transferred from facility 8 during the outbreak. A fifth outbreak (n=3) occurred in facility 9 in November 2013, an epidemiological link to other 027 outbreaks was not found. Facility 4 had several 027 cases (n=5) at the IC unit since October 2013, without fulfilling the outbreak definition.

MLVA

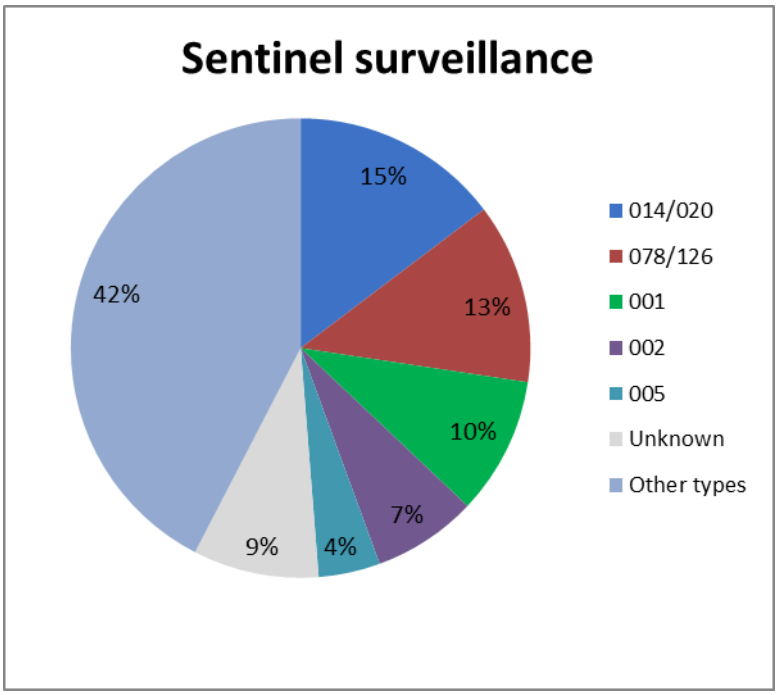
Multiple-Locus Variable number tandem repeat Analysis was used to study the relatedness between the 027 outbreak strains (Figure 5). Results showed that the outbreak strain from facility 3 was different (>10 STRD) from the genetically related outbreak strains of facility 4, 8, 9 and 10 (≤10 STRD). The 027 strain causing outbreaks in facility 4, 8, 9 and 10 transmitted overall to at least seven hospitals and seven nursing homes in different areas of the country.

Table 4: Number of isolates sent to the Reference Laboratory for ad hoc typing per location. Period: May 1st 2013 – May 1st 2014. Hospitals that participate in the sentinel surveillance are not included. Facility 3 started participating in the surveillance since August 2013; strains send before that time are included in this table. 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.

Location	Samples*		Sample	*C. difficile*		Most common type			2nd most common type		
	N	%	type	N	%		N	%		N	%
1	27	16%	Isolates	25	93%	001	8	32%	078/126	6	24%
2	24	14%	Isolates	22	92%	014/020	4	18%	001	3	14%
3(=U)	23	13%	Isolates	23	100%	027	16	70%	014/020	2	9%
4	19	11%	Isolates	19	100%	027	5	26%	015	4	21%
5	18	10%	Isolates/feaces	17	94%	005	3	18%	078/126	3	18%
6	18	10%	Isolates	17	94%	027	10	59%	078/126	2	12%
7	13	7%	Faeces	12	92%	081	3	25%	001	2	17%
8	10	6%	Isolates	10	100%	027	10	100%	-	-	-
9	5	3%	Unknown	3	60%	027	3	100%	-	-	-
10	4	2%	Faeces	4	100%	027	3	75%	Unknown	1	25%
11	3	2%	Faeces	1	33%	027	1	100%	-	-	-
12	2	1%	Isolates	2	100%	017	1	50%	078/126	1	50%
13	2	1%	Isolates	2	100%	014/020	1	50%	078/126	1	50%
14	2	1%	Unknown	1	50%	Unknown	1	100%	-	-	-
15	1	1%	Faeces	0	0%	-	-	-	-	-	-
16	1	1%	Faeces	1	100%	014/020	1	100%	-	-	-
17	1	1%	Faeces	1	100%	070	1	100%	-	-	-
18	1	1%	Isolate	1	100%	014/020	1	100%	-	-	-
	174	**100%**		**161**	**93%**	**027**	**51**	**32%**	**078/126**	**18**	**11%**

* Isolates and faecal samples

Figure 4: Proportions of five most frequent encountered PCR ribotypes. Data from the sentinel surveillance and from the ad hoc typing are displayed separately. Period: May 1st 2013 – May 1st 2014. The category 'other types' consists of 51 different types in the Sentinel surveillance data and 22 different PCR-ribotypes in the ad hoc typing data.



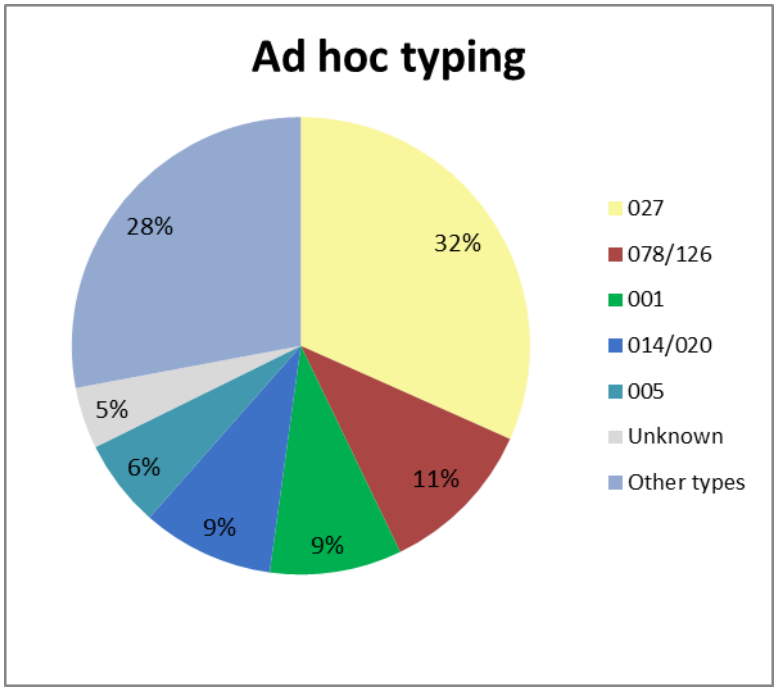
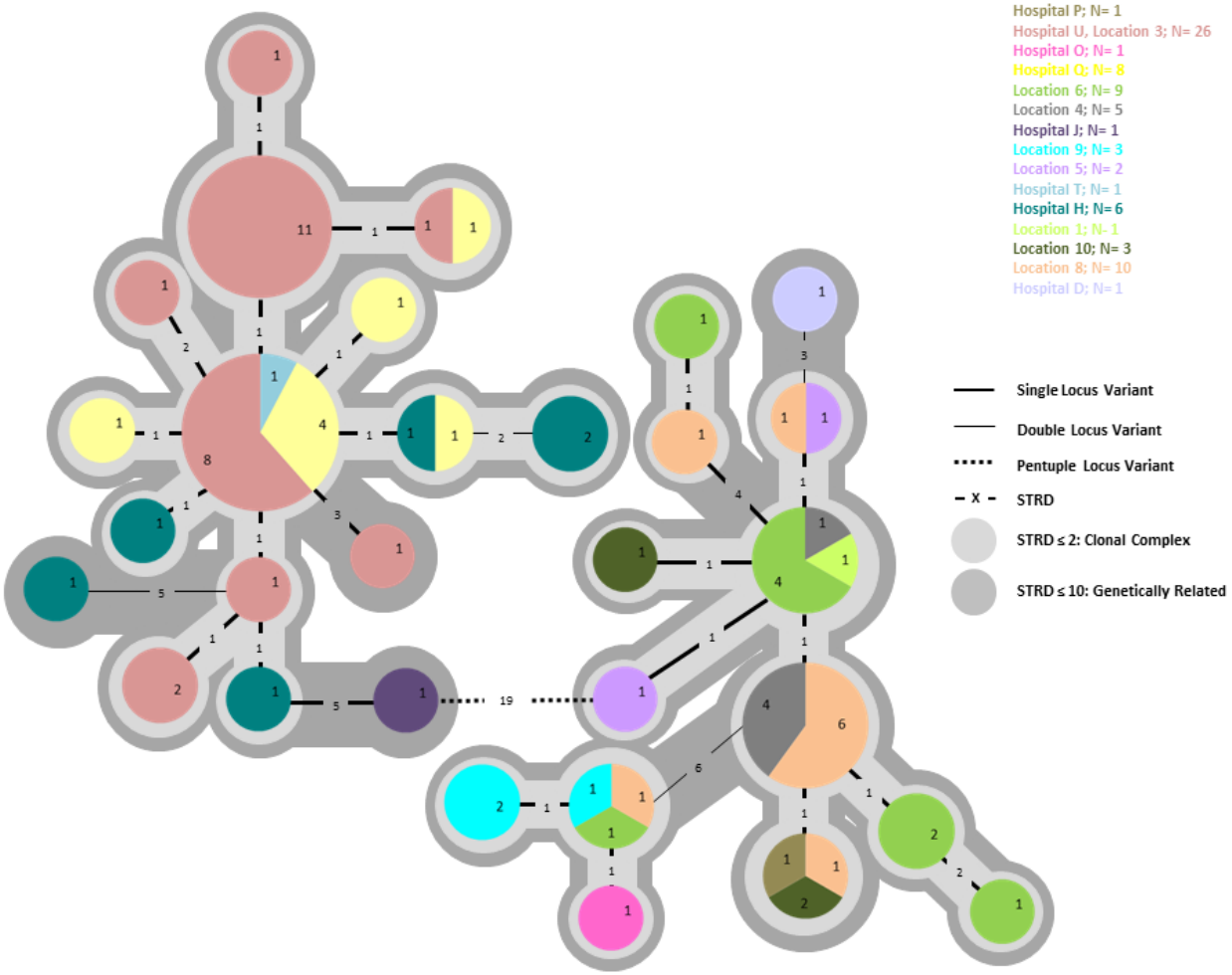


Figure 5: Multiple-Locus Variable number tandem repeat Analysis (MLVA) was used to study the relatedness between the 027 outbreak strains. The figure depicts the minimum spanning tree; each circle contains 100% identical MLVA types, and the numbers in the circles/pies represent the number samples from 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). Results showed that the outbreak strain from hospital U/facility 3 was different (>10 STRD) from the genetically related strains of facility 4, 8, 9 and 10 (≤10 STRD). As location 6 sent strains from different nursing homes, this strain transmitted overall to at least seven hospitals and seven nursing homes in different areas of the country.



Conclusions and recommendations

- The Reference laboratory is now capable to recognize 174 different PCR ribotypes, including five new ribotypes (154, 213, 181, 387, 430, 248, 293, 171, 186, 204, 104). This year, 93% of the samples submitted for ad hoc typing and 88% of the samples submitted by participants of the sentinel surveillance contained *Clostridium difficile.*
- Concerning diagnostic testing, we observed a switch from using toxin enzyme immunoassays to more sensitive diagnostic tests, such as assays detecting glutamate dehydrogenase and PCR. In 2014, an official updated guideline on CDI diagnostic will be published by our institute on behalf of the ESCMID.
- The results of the sentinel surveillance in 20 hospitals revealed that the mean incidence was 16.2 per 10.000 hospital admissions (varying from 4 to 30 per 10.000 admissions). This incidence is comparable to the incidence of 15 per 10.000 admissions that was reported in the recent years.
- The most frequent encountered PCR ribotypes included ribotype 014/020 (14%), the closely related ribotypes 078 and 126 (13%), and ribotype 001 (8%).The proportion of ribotype 001 is decreasing over the last years. Ribotype 027 was equally prevalent as previous years (3%). Within most hospitals, predominant ribotypes change in time.
- 21% of 693 patients (n=143) had severe CDI. After 30 days, 2% of the patients (n=15) were admitted to the ICU and 11% died (n=70), of which 18 patients contributable to CDI. No deaths solely due to CDI were reported this year, and none of the patients needed surgery as a consequence of their CDI.
- It is estimated that more than 3000 hospitalized patients annually will develop CDI of which 120 will succumb contributable to CDI (with 91 hospitals and 2,017,000 hospital admissions per year, according to data from the Dutch Hospital Data and a mortality of 3.7% as reported by Hensgens et al.).[24] [25] In these estimations, the impact of CDI in other healthcare facilities than hospitals was not included. Therefore, the true number of patients with CDI admitted to health care facilities will be higher.
- Between May 1st 2013 and May 1st 2014, no outbreaks were reported by surveillance hospitals.
- In the period between May 1st 2013 and May 1st 2014, 18 facilities sent strains to the Reference Laboratory for ad hoc typing, because of outbreaks or severe cases of CDI. Ribotype 027 was the predominant ribotype, found in 32%, followed by ribotype 078/126 (11%).
- Seven outbreaks were observed; five of these outbreaks were caused by ribotype 027 and two by ribotype 001. Two hospitals needed extensive infection control measures, including restriction of quinolone use, to control 027 outbreaks. The outbreak strain from one of these hospitals was found in seven other hospitals and seven nursing homes, examined by MLVA.
- Therefore, alertness for transmission of ribotype 027 yet again is needed.

[24] Hensgens et al. All-cause and disease-specific mortality in hospitalized patients with *Clostridium difficile* infection: a multicenter cohort study, Clin Infect Dis. 2013 Apr;56(8):1108-16.doi: 10.1093/cid/cis1209. Epub 2013 Jan 8.
[25] Kengetallen Dutch Hospital Data, Utrecht, February 2013. www.dutchhospitaldata.nl

Output

Completed PhD thesis May 2013-May 2014

M.P. Hensgens. Risk factors, course and outcome of *Clostridium difficile* infections. 15 October 2013, Leiden.

S. Debast. *Clostridium difficile* infection; the role of antibiotics in outbreak control, epidemiology and treatment. 13 February, 2014, Leiden.

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 May 2013 – May 2014 related to the reference laboratory

Obuch-Woszczatyński P, Lachowicz D, Schneider A, Mól A, Pawłowska J, Ożdżeńska-Milke E, Pruszczyk P, Wultańska D, Młynarczyk G, Harmanus C, Kuijper EJ, van Belkum A, Pituch H. Occurrence of *Clostridium difficile* PCR-ribotype 027 and it's closely related PCR-ribotype 176 in hospitals in Poland in 2008-2010. Anaerobe. 2014;28C:13-17.

Arvand M, Vollandt D, Bettge-Weller G, Harmanus C, Kuijper EJ*; Clostridium difficile* study group Hesse. Increased incidence of *Clostridium difficile* PCR ribotype 027 in Hesse, Germany, 2011 to 2013. Euro Surveill. 2014;19(10). pii: 20732

Álvarez-Pérez S, Blanco JL, Martínez-Nevado E, Peláez T, Harmanus C, Kuijper E, García ME. Shedding of *Clostridium difficile* PCR ribotype 078 by zoo animals, and report of an unstable metronidazole-resistant isolate from a zebra foal (Equus quagga burchellii). Vet Microbiol. 2014: 14;169:218-22.

van Nood E, Keller JJ, Kuijper EJ, Speelman P. New treatment options for infections with *Clostridium difficile*. Ned Tijdschr Geneeskd. 2013;157(48):A6580.

Hensgens MP, Kuijper EJ. *Clostridium difficile* infection caused by binary toxin-positive strains. Emerg Infect Dis. 2013;19:1539-40.

Bauer MP, Farid A, Bakker M, Hoek RA, Kuijper EJ, van Dissel JT. Patients with cystic fibrosis have a high carriage rate of non-toxigenic *Clostridium difficile*. Clin Microbiol Infect. 2013 Nov 1. doi: 10.1111/1469-0691.12439.

Álvarez-Pérez S, Blanco JL, Peláez T, Astorga RJ, Harmanus C, Kuijper E, García ME. High prevalence of the epidemic *Clostridium difficile* PCR ribotype 078 in Iberian free-range pigs. Res Vet Sci. 2013;95:358-61.

Hensgens MP, Dekkers OM, Goorhuis A, LeCessie S, Kuijper EJ. Predicting a complicated course of *Clostridium difficile* infection at the bedside. Clin Microbiol Infect. 2014 ;20:O301-8.

Debast SB, Bauer MP, Kuijper EJ; Committee. European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for *Clostridium difficile* infection. Clin Microbiol Infect. 2014;20 Suppl 2:1-26.

Keessen EC, Harmanus C, Dohmen W, Kuijper EJ, Lipman LJ. *Clostridium difficile* infection associated with pig farms. Emerg Infect Dis. 2013;19:1032-4.

Keessen EC, Hensgens MP, Spigaglia P, Barbanti F, Sanders IM, Kuijper EJ, Lipman LJ. Antimicrobial susceptibility profiles of human and piglet *Clostridium difficile* PCR-ribotype 078. Antimicrob Resist Infect Control. 2013;2:14. doi: 10.1186/2047-2994-2-14.

Obuch-Woszczatyński P, Dubiel G, Harmanus C, Kuijper E, Duda U, Wultańska D, van Belkum A, Pituch H. Emergence of *Clostridium difficile* infection in tuberculosis patients due to a highly rifampicin-resistant PCR ribotype 046 clone in Poland. Eur J Clin Microbiol Infect Dis. 2013;32:1027-30.

Debast SB, Bauer MP, Sanders IM, Wilcox MH, Kuijper EJ; ECDIS Study Group. Antimicrobial activity of LFF571 and three treatment agents against *Clostridium difficile* isolates collected for a pan-European survey in 2008: clinical and therapeutic implications. J Antimicrob Chemother. 2013;68:1305-11.

Knetsch CW, Lawley TD, Hensgens MP, Corver J, Wilcox MW, Kuijper EJ. Current application and future perspectives of molecular typing methods to study *Clostridium difficile* infections. Euro Surveill. 2013;18(4):20381.

van den Berg RJ, Bakker D, Kuijper EJ. Diagnosis of *Clostridium difficile* infection using real-time PCR. Methods Mol Biol. 2013;943:247-56. doi: 10.1007/978-1-60327-353-4_16.

Presentations and posters May 2013 – May 2014

NVMM, voorjaarsvergadering 15 en 16 April, 2014, Papendal.

(Oral) O023; SM van Dorp, DW Notermans, G Kampinga, A Buiting, ECDIS-network, EJ Kuijper. Changes of the epidemiology of CDI in the Netherlands and Europe.

(Oral) O024; Sylvia Debast. Update of European guidelines for the treatment of *Clostridium difficile* infection (CDI)

(Oral) O026; Wilco Knetsch. Whole genome sequencing revealed transmission of *Clostridium difficile* between humans and farm animals in the Netherlands.

23nd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), 10-13 May, 2014, Barcelona, Spain.

(Poster) P0754; van Dorp SM, Kola A, Behnke M, Gastmeier P, Schmid D, Hajdu A,. Kampinga GA, Suetens C, Kuijper EJ. European *Clostridium difficile* infection surveillance network; Towards a Europe-wide *C. difficile* infection surveillance

(Poster) P0755; van Dorp SM, Kola A, Behnke M, Gastmeier P, Schmid D, Hajdu A, Kampinga G, Suetens C, Kuijper EJ, European Clostridium difficile infection surveillance network; *C. difficile* infections in acute care hospitals – results of the pilot study of a European surveillance initiative

(Poster) P0759; Pituch H, Lachowicz D, Obuch-Woszczatynsk P, Mlynarczyk G, Harmanus C, Kuijper E. Polish surveillance programme of *Clostridium difficile* infections reveals persistently high prevalence of *Clostridium difficile* type 027.

(Poster) P0779; Beran V, Kuijper E, Harmanus C, Sanders I, van Dorp S, Janeckova J, Kopecky O, Barekova L, Chmelar D, Ciznar. Emergence of *Clostridium difficile* type 176 in Czech Republic.

(Poster) P0792; Sanders IM, Harmanus C, Debast S, Kuijper EJ. Antibiotic susceptibility of surotomycin and five other antibiotics against *Clostridium difficile* isolates, collected at a pan-European survey in 2008 (n=119).

(Oral) O006; van Dorp SM; Notermans DW, de Greeff S, Kuijper EJ. Dutch sentinel surveillance for *Clostridium difficile* infections shows stable hospital incidence since 2009, but a re-emergence of type 027.

(Oral) O033; Knetsch W, Connor T, Mutreja A, Sanders I, Van Dorp S, Lipman L, Keessen L, Corver J, Kuijper E, Lawley T. Spread of *Clostridium difficile* between humans and farm animals in the Netherlands revealed by whole genome sequencing

(Poster) P0251; Debast SB; Bauer MP; Kuijper EJ, European Society of Clinical Microbiology and Infectious Diseases (ESCMID): update of the treatment guidance document for *Clostridium difficile* infection (CDI).

(Abstract book) R476; Krutova M, Matejkova J, Kuijper E, Harmanus C, Nyc O. Emergence of *Clostridium difficile* 027-like PCR ribotype 176 in the Czech Republic (2012-2013).

Invited presentations May 2013 – May 2014

27 May, 2013; Frankfurt. "Symposium Clostridium difficile – Aktuelles zur Epidemiologie, Diagnostik und Therapie". (Organised by PD Dr. Lutz von Müller, Institut für Medizinische Mikrobiologie und Hygiene, Konsiliarlabor für *C. difficile*, Universitätsklinikum des Saarlandes, Homburg/Saar)

Ed J. Kuijper, M. Hensgens and D. Notermans. *Clostridium difficile* outside of the hospital: Community associated and zoonotic infections.

29, 30 August 2013; Society for General Microbiology, Ulster, Ireland
Ed J. Kuijper, Len Lipman and Daan Notermans. "*Clostridium difficile* Type 078 in humans, pigs and food".

5 September, 2013; Congres Infectieuze bedreigingen, Ede.
Ed J. Kuijper. "*Clostridium difficile*: de rol van antibiotica en fecestransplantatie".

22-25 September, 2013; Rostock, Germany University of Rostock. 65th Annual Meeting, German Society for Hygiene and Microbiology (DGHM), Annual Meeting of the German Society for Infectiology.
Ed J. Kuijper, S. van Dorp, D. Notermans. "Epidemiology and prevention of *Clostridium difficile* infections".

5 December, 2013; Dublin. Focus on Infection, 2013 Royal College of Physicians.
Ed J. Kuijper. "*Clostridium difficile* Infection: New Management Strategies".

12 December 2013, Evoluon, Eindhoven; Dr. Falk Pharma Benelux B.V and MUMC; Chronic diarrhea in adults; underestimated and emerging causes.
Ed J. Kuijper, S. van Dorp and D. Notermans. "Is *Clostridium difficile* emerging?"

30 January, 2014; Utrecht University, KNVM; Typeren met beleid.
Ed J. Kuijper, S. van Dorp and D. Notermans. “Standaard typering en uitbraak typering van *Clostridium difficile”.*

Organization of Workshops and congress sessions.

Session at NVMM voorjaarsvergadering, 15-16 April 2014; New insights in epidemiology and treatment of *Clostridium difficile* infections (CDI).

Session at ECCMID 2014, Barcelona. Emergence of *Clostridium difficile* infections outside healthcare facilities.