Seventh Annual Report of the National Reference Laboratory for *Clostridium difficile* and results of the national surveillance May 2012 to May 2013

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Content

Abstract

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 *Clostridium difficile* at the Leiden University Medical Center (LUMC). This “ad hoc typing” was offered to all microbiological laboratories in the Netherlands. Medical microbiologists in the Netherlands were requested to send *Clostridium difficile* samples from patients with severe CDI and from outbreaks to the Reference Laboratory Surveillance; resulting in recognition of new emerging *C. difficile* PCR ribotypes, such as Type 078, which was also found increasingly in other European countries. As of May 2009, a sentinel surveillance was added with continuous monitoring of CDI in approximately 20 hospitals collecting a minimum of clinical and epidemiological and characterization of *C. difficle* isolates at the LUMC. The Reference Laboratory is now able to recognize 164 PCR ribotypes. In 2012, two new PCR ribotypes were added to the Reference Laboratory library.

In the period between May 1st 2012 and May 1st 2013, 296 samples from 17 healthcare facilities and laboratories in the Netherlands were investigated at the Reference Laboratory in Leiden for ad hoc typing. PCR ribotype 027 was more frequently found (20%) compared to 2011-2012 (15%). This is attributable to a large outbreak encompassing at least 69 isolates from a hospital and surrounding nursing homes in the eastern part of the Netherlands. We also found isolates that were related to this particular strain in three hospitals using MLVA, of which two in another part of the country.

The results of the sentinel surveillance in the period May 2012-May 2013 in 19 hospitals showed that the mean incidence of CDI was 14.7 per 10,000 hospital admissions, varying from 5 to 27 per 10,000 admissions. This incidence is similar as the incidence of 15 per 10,000 admissions that was reported the recent two years. The most frequent encountered PCR ribotypes were Type 014 (16%), Type 001 (14%), Type 078 (12%), Type 002 (6%) and Type 005 (5%). Of all (n=911) *C. difficile* isolates, 28 (3%) belonged to Type 027; no Type 027-like PCR ribotypes (016, 036,176) were found. A total of 150 patients (25%) had severe CDI. Within 30 days, six patients had been admitted to the ICU and two underwent surgery as a consequence of CDI; 56 patients with CDI (11%) died. Twelve deaths were attributable or contributable to CDI. Extrapolating the data of sentinel surveillance to all hospitals in the Netherlands, it is estimated that more than 2900 hospitalized patients annually will develop CDI. 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 healthcare facilities will be higher.

We conclude that Type 027 is re-emerging related to outbreaks in two healthcare facilities in different parts of the country. The proportion of Type 027 in the ad hoc typing increased to 20%, however, the proportion of Type 027 in the sentinel surveillance is stable at 3%.

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, 164 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.[1]

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 *Clostridium 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 Type 027 is associated with a higher morbidity and mortality and has tendency to relapse more frequently.

As of 2005, outbreaks with Type 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 typing service for *Clostridium 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 *Clostridium 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 PCR ribotypes that were 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.

The 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.[2] [3] [4] Furthermore, a decrease in Type 027 was noted, together with an increase of the more virulent Type 078.[15] This type has also been found as an increasing type in Europe and is currently the third most frequently found type European wide.[6] Interestingly, *C. difficile* Type 078 has also been recognized as one of the most important agents of piglet-associated diarrhoea[7], and humans and swine carry genetically identical strains based on whole-genome sequencing.[8]

In order to study the incidence of CDI in an endemic situation, a new sentinel surveillance started in May 2009. Nineteen to twenty hospitals participated in this surveillance and introduced a continuous monitoring of CDI. This is the fourth annual report that provides an overview of the two types of surveillance conducted in the Netherlands. The previous three reports showed that the incidence in the Netherlands is stable at 15 per 10,000 admissions and the most frequently found types are 001, 078 and 014.[9] In this annual report we describe the situation in the Netherlands between May 1st 2012 and May 1st 2013.

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[1] 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.

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

[3] 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

[4] 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.

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

[6] Bauer MP, Notermans DW, van Benthem BH et al. J; ECDIS Study Group. *Clostridium difficile* infection in Europe: a hospital-based survey. Lancet. 2011;377:63-73.

[7] Debast SB, van Leengoed LA, Goorhuis A, Harmanus C, Kuijper EJ, Bergwerff AA. *Clostridium difficile* PCR ribotype 078 toxinotype V found in diarrhoeal pigs identical to isolates from affected humans. Environ Microbiol. 2009;11:505-11

[8] Knetsch W, Whole-genome sequencing reveals potential interspecies transmission of *Clostridium difficile,* type 078 oral presentation NVMM 16th of april 2013.

[9] Sixth and Fifth Annual Report of the National Reference Laboratory for Clostridium difficile and results of the sentinel surveillance available at: http://www.rivm.nl/Onderwerpen/Ziekten_Aandoeningen/C/Clostridium_difficile

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 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 fecal 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.

Ad 2. All fecal 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, to CIb and are 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 or twice 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.[10] [11] 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.

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

[11] 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 feces 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, 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 feces 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 pretreatment. 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*.[12] All *C. difficile* strains are further investigated by PCR-ribotyping.[13] The presence of *tcdA*, *tcdB* and binary toxin genes is investigated according to standardized techniques.[14] [15] [16] Deletions in *tcd*C were determined by PCR using in-house designed primers.[17]

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. Since May 2006, all 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 fecal 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.[18] [19]

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[12] Paltansing et al. Characteristics and incidence of *Clostridium difficile*-associated disease in The Netherlands, 2005. Clin Microbiol Infect 2007;13:1058-64

[13] 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

[14] Goncalves et al. Prevalence and characterization of a binary toxin (actin-specific ADP-ribosyltransferase) from *Clostridium difficile*. J Clin Microbiol 2004;42:1933-9

[15] Kato et al. Identification of toxin A-negative, toxin B-positive *Clostridium difficile* by PCR. J Clin Microbiol 1998;36:2178-82

[16] 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

[17] Kuijper et al. *Clostridium difficile* ribotype 027, toxinotype III, the Netherlands. Emerg Infect Dis 2006; 12:827-30

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

[19] 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

The sentinel surveillance for *Clostridium difficile* infections was initiated in May 2009 to obtain systematic and representative incidence data and to identify new circulating ribotypes in the Netherlands. This report describes the results of the current 19 participating hospitals, including one new participant since January 2013. Both academic centres (n=5) and general hospitals (n=14) are included and distributed all over the Netherlands. The geographical location of the participating centres is displayed in figure 1. However, similar to the previous years, due to organisational problems not all hospitals were actively contributing epidemiological data, but isolates were still collected and send to the Reference Laboratory for PCR ribotyping. Between May 1st 2012 and May 1st 2013, the 19 participating centres submitted 1020 samples, from which 911 (89,3%) yielded *C. difficile.*

Circulating PCR ribotypes (Figure 4) Type 014 was the most frequently found type, isolated in 145 of the 911 isolates (15.9%). Type 001 was found in 131 isolates (14.4%, 95% CI 12,3-16,8), Type 078 in 110 isolates (12.1%), Type 002 in 50 isolates (5.5%) and Type 005 in 46 isolates (5.0%). Twenty-eight isolates were identified as Type 027 (3.1%) and no type 027-like PCR ribotypes (016, 036,176)[20] were found. The closely related ribotypes 078 or 126 (Knetsch, preliminary whole genome sequencing data), were found in 132 isolates (14.5%). Of 61 isolates (6.7%) the PCR ribotype pattern was not recognized in our database, which is less than last year (9%). The results stratified per participating centre are displayed in Table 2a.
Compared to the previous years, some changes were noticed. The proportion of Type 001 did slightly decrease (2010-2011 20%; 95% CI 17,7-22,9, 2011-2012 17%; 95% CI 14,2-19,2) and of Type 014 did slightly increase, though not statistically significant (2010-2011 95% CI 10,9-15,3; 2011-2012 95% CI 11,3-15,9). The proportions of Type 078 (2010-2012 12%),Type 002 (2010-2012 6%), and Type 005 (2011-2012 5.1%) were stable. The eight most frequently found PCR ribotypes compared to May 2011-May 2012 are illustrated in Table 2b.

Table 3 also depicts the diagnostic tests used by the participating laboratories to diagnose CDI. In comparison to 2012, no major changes were reported. Six hospitals used PCR or LAMP (Loop-Mediated Isothermal Amplification) to screen for *C. difficile*, two hospitals used an enzyme immunoassay for both GDH and toxins. The remaining eleven hospitals used two other toxin enzyme immunoassays, of which one hospital also performed *C. difficile* culture for screening.

Outbreaks in participating hospitals

Between May 1st 2012 and May 1st 2013, one large outbreak caused by Type 027 was observed in a participating hospital to the sentinel surveillance, and two small outbreaks were suspected. An suspected outbreak was defined if >2 isolates of the same type were found less than 7 days apart in one hospital EITHER on the same department of the hospital OR accompanied with an increased CDI monthly incidence within the hospital. A large outbreak was defined as >4 isolates of the same type found less than 7 days apart in one hospital EITHER on the same department of the hospital OR accompanied with an increased CDI monthly incidence within the hospital.

One hospital in the western part of the Netherlands (Table 2a Hospital D) had an outbreak associated with Type 027 encompassing at least 4 patients from ward of internal medicine and 3 patients from other wards. MLVA (Multi-Locus Variable number tandem repeat Analysis) clearly demonstrated a clonal spread (Figure 2). During this outbreak, one patient was infected by a clindamycin resistant Type 027 isolate, resembling Type 027 strains in the outbreak region in the eastern part of the Netherlands (See results Ad hoc typing). One hospital participating in the sentinel surveillance in the outbreak region in the eastern part of the Netherlands (Table 2a Hospital C) reported an increased incidence of CDI in May-June 2012 (22-34 per 10.000 admissions), including

[20] 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.

five confirmed Type 027 cases and six Type 001 cases. However, according to our definition there was no outbreak and a clear epidemiological link between the cases caused by the same PCR ribotype was not found. Type 027 was sporadically found in five other participating hospitals, but did not cause any outbreak.

In one hospital (Table 2a Hospital A), *C. difficile* Type 001 caused an outbreak in August 2012 (n=4) on different departments with a monthly incidence of 34 per 10.000 admissions. The rest of the year, Type 001 was predominantly (n=31 incl. the outbreak cases) found on various departments, indicating a hyperendemicity. Further, one hospital (Table 2a Hospital Q) had a small outbreak of three patients with CDI due to Type 015 cases in May-June 2012.

Incidence in participating hospitals

The incidence of CDI was measured using the data of the months in which patients were actively included, and are shown in Table 3. Fourteen hospitals (74%) participated actively during the whole study period (12 months of active participation). Among hospitals that did not administer data during the whole surveillance period, two hospitals included patients for only four months, and two hospitals were one to three months behind with administrating data. One participant started in January 2013 and participated for four months.

Data about number of admissions and number of admission-days were known for all participating hospitals (personal communication or the most recent annual report of the hospital). The mean incidence was 14.7 per 10,000 hospital admissions (varying from 5 to 27 per 10,000 admissions), comparable to the incidence of 15 per 10,000 admissions that was reported in 2011 and 2012.[9]

Demographical and clinical data of the sentinel surveillance

Demographical and clinical characteristics were collected from all patients that were included in the sentinel surveillance. Of the 704 patients included, 340 were male (48.3%) and 364 were female (51.7%). The mean age was 67 years (SD 19.6), varying from 3 to 97 years. 3.7% Of the patients was younger than eighteen. Of 669 patients the location of onset of complaints was known (*Table 1a*; 249 (37%) patients had community-onset CDI, the remaining 420 patients (63%) were admitted in a healthcare centre when the diarrhoea started. These patients were most frequently admitted to a hospital (n=377;90%) at the department of internal medicine (n=109; 29%) and general surgery (n=47; 12%). Twenty-two patients were admitted to the intensive care unit (6%). Further, 21 patients (3%) had their onset of diarrhoea in a nursing home, and another 22 patients (3%) in another healthcare facility.

Most patients used antibiotics prior to the start of diarrhoea (416/573; 73%). In the eight weeks prior to the start of this episode of diarrhoea, 81 patients (81/341; 24%) had an episode of CDI. A total of 150 patients (25%) had severe CDI, defined as bloody diarrhoea and/or diarrhoea with hypovolemia or hypo-albuminaemia (<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 course of the disease was known for 517 patients. 453 patients (88%) had an uncomplicated course of their CDI infection. After 30 days, 6 patients (1.2%) were admitted to the ICU as a consequence of CDI, 2 patients (0.4%) needed surgery as a consequence of their CDI and 56 patients with CDI (11%) died. Twelve deaths (2.3%) were contributable to CDI. Three of these patients had CDI due to Type 078 and another two patients had CDI due to Type 005. Other types involved in contributable death were Type 001, 002, 014, 057, and 095. Of two CDI-related deaths, there was no *C. difficile* culture available for PCR ribotyping. The outcome of CDI patients stratified per Type 027, Type 078, and other types is shown in Table 1b.

***Table 1a*:** Location of onset of complaints, reported for 669 patients.

Location	Number of patients	Percentage
Hospital	377	56%
Nursing home	21	3%
Other health-care facility	22	3%
Community	249	37%
Total	669	100%

***Table 1b*:** Outcome of CDI patients, stratified per PCR ribotype 027, 078 and other ribotypes.

	PCR ribotype					
	027		**078**		**other types**	
	N	**%**	**N**	**%**	**N**	**%**
Severe diarrhoea	4/5	80	19/58	33	104/534	19
Complicated course	1/4	25	11/44	25	44/373	12
Mortality contributable to CDI	0/4	0	3/44	7	7/373	1.9

***Figure 1*:** Participating hospitals of the sentinel surveillance.

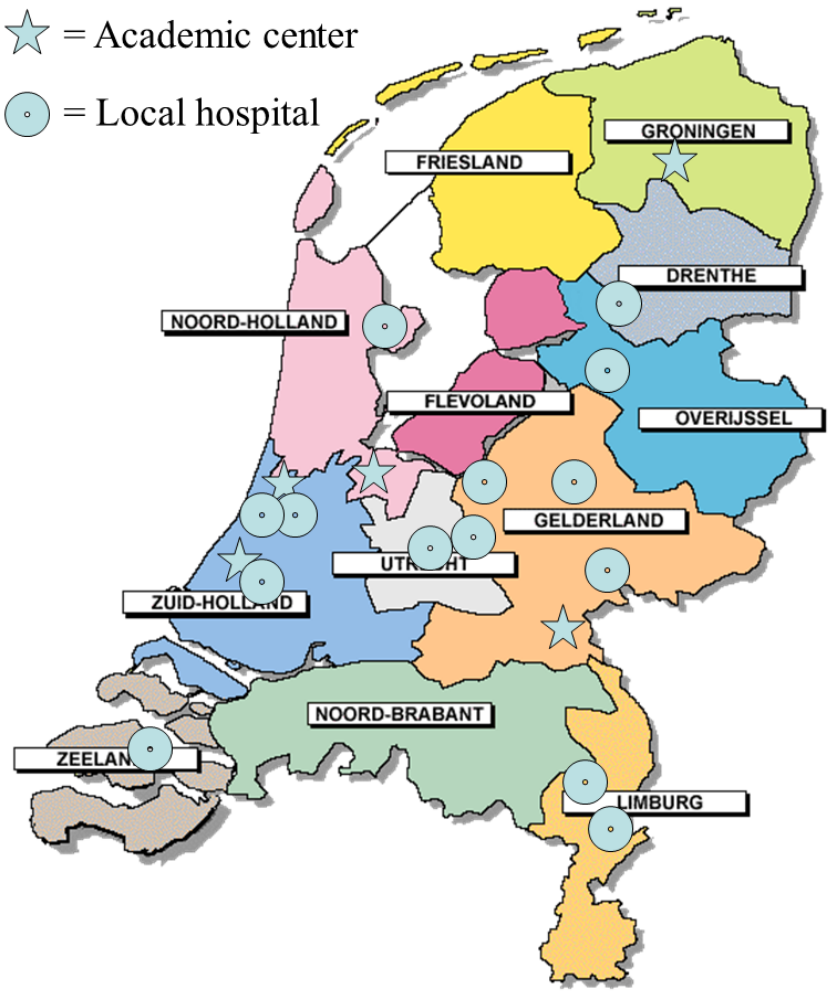


Figure 2: MLVA (Multi-Locus Variable number tandem repeat Analysis) which demonstrates the outbreak in hospital D caused by PCR ribotype 027 in May 2013 in the western part of the Netherlands, and a second introduction of a non-related clindamycin resistant 027 strain (D4) related to the 027 strains that were found in Location 2 and hospital C the eastern part in the Netherlands in the same month. D3s1 and D3s2 are two strains from the same patient collected with a time interval of twenty days.

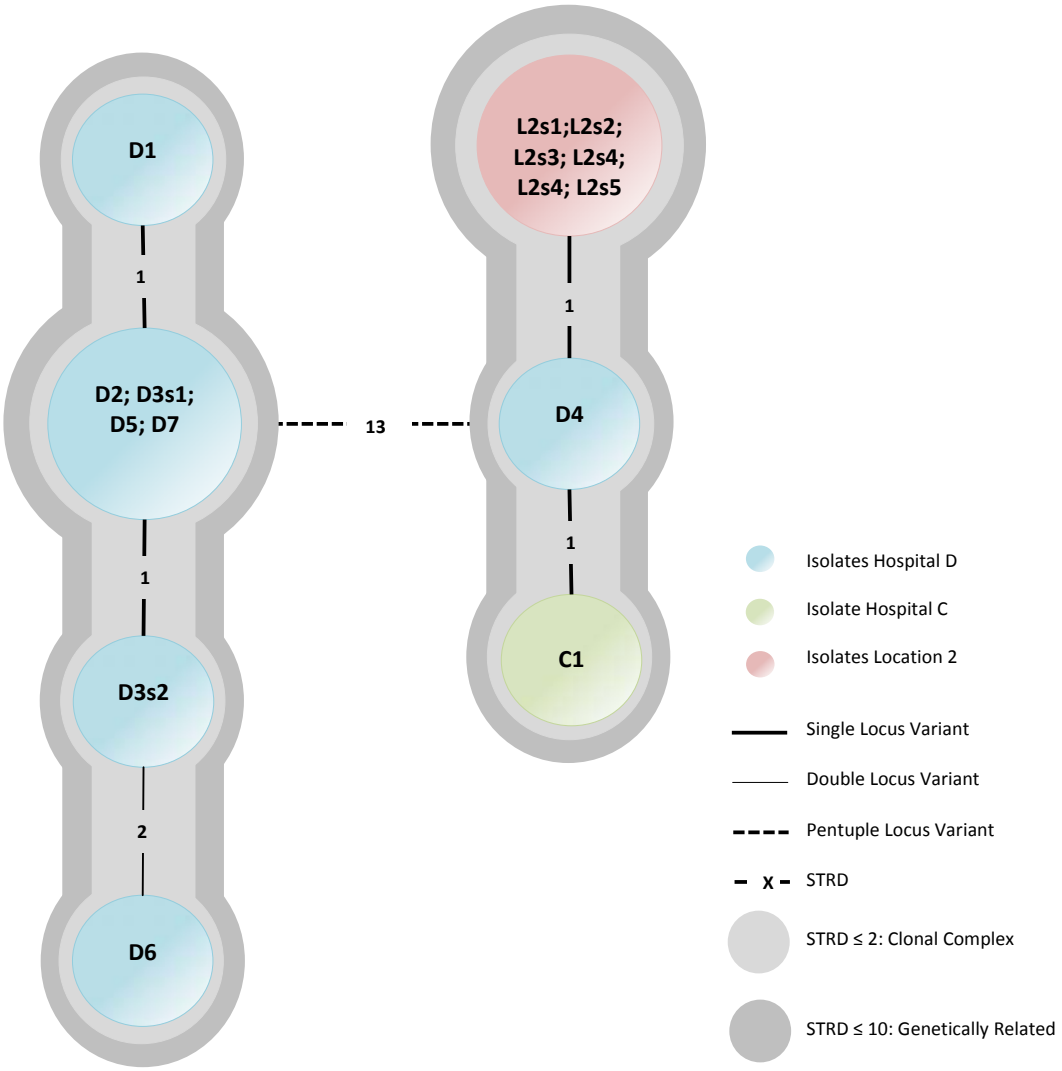


Table 2a: Isolates submitted for PCR ribotyping by hospitals participating in the sentinel surveillance. Period: May 1st 2012 – May 1st 2013. If different PCR ribotypes were equally frequently found, the PCR ribotype with the lowest number is first reported.

Hospital	Samples		Sample	*C. difficile*		Most common type			2nd most common type		
	N	%	type*	N	%		N	%		N	%
A	130	12,7%	Isolates	126	96,9	001	57	45	014	11	9
B	39	3,8%	Isolates	35	89,7	078#	8	23	unknown	5	14
C	124	12,2%	Isolates	111	89,5	001	18	16	014	17	15
D	55	5,4%	Isolates	53	96,4	014#	8	15	027	8	15
E	41	4,0%	Faeces	30	73,2	014	6	20	015	4	13
F	21	2,1%	Isolates	16	76,2	014	5	31	002	2	13
G	68	6,7%	Isolates	68	100,0	078	13	19	unknown	8	12
H	48	4,7%	Isolates	46	95,8	001	12	26	014	8	17
I	16	1,6%	Isolates	16	100,0	unknown#	3	19	005	2	13
J	68	6,7%	Faeces	54	79,4	014	14	26	001	8	15
K	6	0,6%	Isolates	6	100,0	002	2	33	014	2	33
L	91	8,9%	Isolates	89	97,8	078#	16	18	014	13	15
M	38	3,7%	Isolates	36	94,7	014	9	25	002	5	14
N	36	3,5%	Faeces	19	52,8	014#	7	37	001	3	16
O	37	3,6%	Faeces	24	64,9	014#	6	25	005	5	21
P	14	1,4%	Isolates	11	78,6	001	4	36	078	2	18
Q	65	6,4%	Faeces	54	83,1	014	11	20	078	11	20
R	16	1,6%	Isolates	12	75,0	001#	5	42	002	1	8
S	107	10,5%	Isolates	105	98,1	078	22	21	014	15	14
Total	**1020**	**100%**		**911**	**89,3**	**014**	**145**	**16**	**001**	**131**	**14**

*Sample type send to LUMC
Different dominant PRC ribotype compared to last year, only reported if >5 samples involved

Table 2b: Eight most frequently found PCR ribotypes of isolates send by hospitals that participate in the sentinel surveillance in the period of May 2012-May 2013, compared to May 2011-May 2012.

	2011-2012		2012-2013	
PCR Ribotype	**N**	**(%)**	**N**	**(%)**
Type 014	112	(13,4)	145	(15,9)
Type 001	138	(16,5)	131	(14,4)
Type 078	103	(12,3)	110	(12,1)
Type 002	48	(5,7)	50	(5,5)
Type 005	43	(5,1)	46	(5,0)
Type 015	25	(3,0)	30	(3,3)
Type 023	14	(1,7)	33	(3,6)
Type 027	26	(3,1)	28	(3,1)
Type unknown	75	(9,0)	61	(6,7)
Other types	251	(30,1)	277	(30,4)
Total	835	(100)	911	(100)

Table 3: Number of patients included in the sentinel surveillance per location and incidence data. Period: May 1st 2012 – May 1st 2013. The incidence data were calculated using only the months of active participation. The primer diagnostic test for CDI is shown per hospital.

Participating hospital	Months of active participation	Patients		Monthly admissions	Monthly patient days	Incidence		Diagnostic test	Incidence per 10.000 admissions 2011-2012
		N	%			per 10.000 admissions	per 10.000 patient days		
A	11	79	11,2%	2659	12121	27,0	5,9	LAMP	28,7
B	12	58	8,2%	2511	16880	19,3	2,9	EIA[1]	22,8
C	8	59	8,4%	3213	14244	23,0	5,2	EIA[1] and culture	23,5
D	12	54	7,7%	2362	10724	19,1	4,2	PCR	14,5
E	12	40	5,7%	3238	13924	10,3	2,4	EIA[2]	13,2
F	4	4	0,6%	1952	8736	5,1	1,1	EIA[2] and PCR	8,5
G	4	14	2,0%	3058	25504	11,4	1,4	EIA[2]	14,4
H	12	29	4,1%	1583	6712	15,3	3,6	LAMP	14,7
I	12	17	2,4%	1553	7583	9,1	1,9	EIA[1]	10,7
J	12	32	4,5%	1763	10484	15,1	2,5	EIA[1]	10,3
K	12	9	1,3%	900	4067	8,3	1,8	EIA[1]	3,2
L	12	73	10,4%	2572	15015	23,7	4,1	PCR	20,7
M	12	31	4,4%	1253	6353	20,6	4,1	EIA[3]	14,4
N	12	23	3,3%	3473	26072	5,5	0,7	EIA[1]	10,7
O	12	21	3,0%	2123	10021	8,2	1,7	EIA[2]	17,3
P	4	14	2,0%	2217	10761	15,8	3,3	EIA[2]	-
Q	12	63	8,9%	3237	17176	16,2	3,1	PCR	17,4
R	12	12	1,7%	856	4098	11,7	2,4	EIA[2]	7,7
S	12	72	10,2%	3931	18313	15,3	3,3	EIA[3]	23,4
		704	100%	44455	238787	14,7	2,9		15,4

LAMP= Loop-Mediated Isothermal Amplification

EIA[1]= Vidas C. difficile Toxin A/B

EIA[2]= Immunocard Toxins A/B, Meridian

EIA[3]= Alere C Diff Quik Chek, Techlab

Results of the ad hoc typing

Hospitals using the Reference Laboratory

In the period between May 1st 2012 and May 1st 2013, 17 healthcare facilities and laboratories in the Netherlands sent samples to the Reference Laboratory in Leiden, for other reasons than for sentinel surveillance, such as severe CDI or suspicion of an outbreak. This number is lower than the previous years, when 26-29 healthcare facilities and laboratories send samples to the Reference Laboratory for ad hoc typing. One of the healthcare facilities started participating in the sentinel surveillance since January 2013; strains submitted before this period were included in the ad hoc typing results. In total, 296 samples were submitted of which approximately 5% consisted of faeces samples instead of isolates submitted by four of the 17 facilities.

Strains sent to the Reference Laboratory

Circulating PCR ribotypes

Of the 296 samples submitted, 82.8% contained *C. difficile.* This percentage is equal to last year. The number of submitted isolates and most common PCR ribotypes, stratified per location, are demonstrated in Table 4. Type 027 was the most commonly found PCR ribotype (19.6%), followed by Type 001 (14.3%), Type 078 (12.7%) and Type 014 (11.0%). The percentage of Type 027 increased compared to last year, but fluctuated in the previous years: 15% in 2011-2012, 26% in 2010-2011, and 4% in 2009-2010. Between 2005 and 2007 the proportion of Type 027 was 12% in 2006-2007 and 21% in 2005-2006. Besides Type 027, the three most frequently PCR ribotypes found were Type 001, 078 and 014. In the previous years, these types were also frequently found.[9 21]

Outbreaks

Since 1st of May 2012, one large and one small outbreaks were observed. An outbreak was defined as >2 isolates of the same type for a small, and >4 isolates for a large outbreak, found less than 7 days apart in one hospital. Data on the departments that were involved or the local incidence rate was not always available.

Two outbreaks caused by PCR ribotype 027 occurred in the same facilities as reported in the previous annual report and comprised all 027 types that were isolated. Most Type 027 (79%, 38/48) samples were submitted by one health-care facility (Table 4 Location 2), which also performs diagnostics for surrounding nursing homes. It was reported that both hospital and nursing home patients were affected by Type 027. During the previous 12 months there was an outbreak from June-November 2012 and in January 2013, but many cases occurred outside these periods. Since January 2011, 69 samples were typed as 027. MLVA (Figure 2) data showed transmission of this outbreak strain to another part of the country. A second outbreak caused by Type 027 was found in another facility (Table 4 Location 1) in November 2012 (n=3). In total, 10 strains of Type 027 were isolated from this facility in the previous 12 months. Again, this facility reported that both hospital and nursing home patients were affected.

Nursing homes Two outbreaks caused by PCR ribotype 027 involved both nursing home (n≥2) and hospitalised patients. One healthcare worker in the affected nursing home developed severe CDI and had to be admitted to the hospital. There is no systematic surveillance for CDI in nursing homes, however, cases of Type 027 and an increased incidence of CDI officially needs to be reported to the local Municipal Service.[22]

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

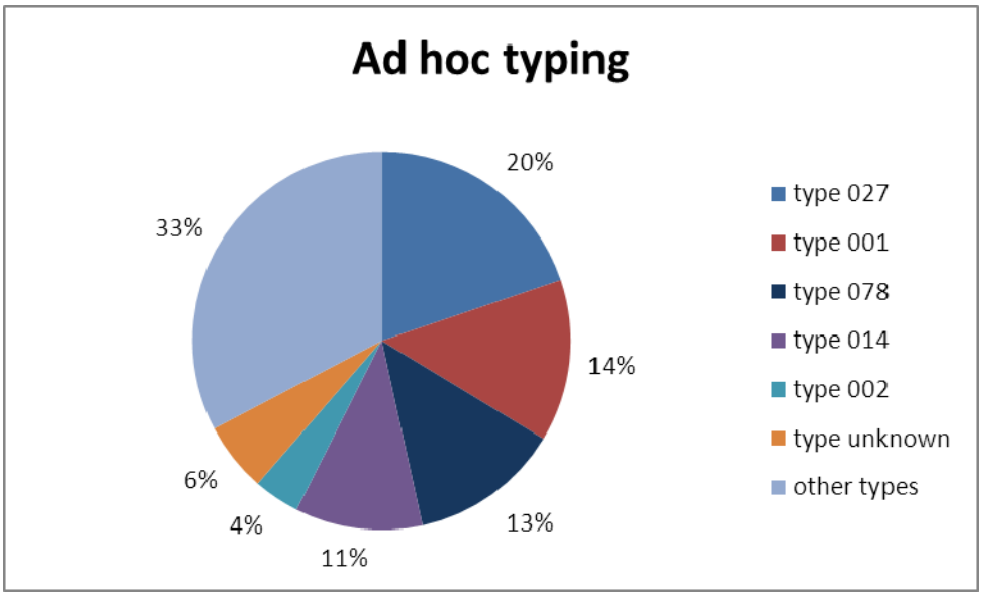
[22] Draaiboek Maatregelen ter preventie en bestrijding *Clostridium difficile* PCR-ribotype 027 – toxinotype III-infectie buiten het ziekenhuis, LCI December 2009

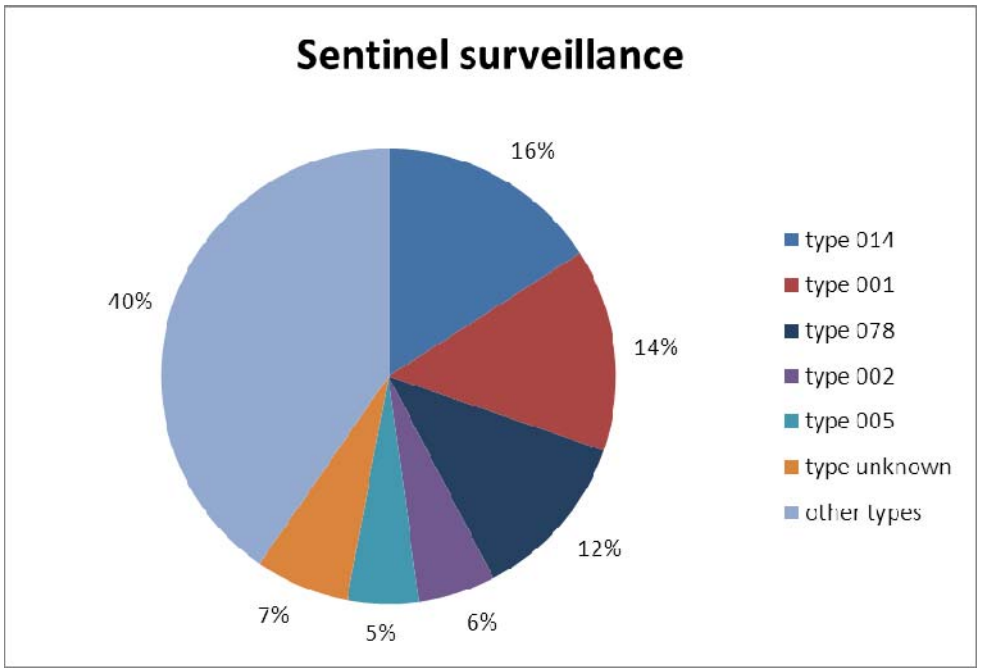
Table 4: Number of isolates sent to the Reference Laboratory for ad hoc typing per location. Period: May 1st 2012 – May 1st 2013. Hospitals that participate in the sentinel surveillance are not included. Facility 4 started participating in the surveillance since January 2013; strains send before that time are included in this table.

Location	Samples*		Sample	*C. difficile*		Most common type			2nd most common type		
	(N)	(%)	type**	(N)	(%)		(N)	(%)		(N)	(%)
1	119	40,2	Isolates	89	75	001	13	11	014	13	11
2	66	22,3	Isolates	62	94	027	38	58	078	9	14
3	44	14,9	Isolates	40	91	001	9	20	078	8	18
4	20	6,8	Isolates	17	85	001	10	50	002	1	5
5	15	5,1	Isolates	12	80	216	3	20	078	2	13
6	8	2,7	Faeces	6	75	014	2	25	078	2	25
7	5	1,7	Isolates	3	60	126	2	40	001	1	20
8	3	1,0	Faeces	2	67	017	1	33	054	1	33
9	3	1,0	Isolates	3	100	014	2	67	001	1	33
10	2	0,7	Faeces	2	100	003	1	50	014	1	50
11	2	0,7	Isolates	2	100	126	1	50	unknown	1	50
12	2	0,7	Isolates	2	100	056	1	50	078	1	50
13	2	0,7	Isolates	2	100	014	2	100	-	-	-
14	2	0,7	Faeces	0	-	-	-	-	-	-	-
15	1	0,3	Isolates	1	100	033	1	100	-	-	-
16	1	0,3	Isolates	1	100	023	1	100	-	-	-
17	1	0,3	Isolates	1	100	095	1	100	-	-	-
Overall	296	100		245	82,8	027	48	19,6	001	35	14,2

* Isolates and faecal samples
**Sample type send to LUMC

Figure 4: Proportions of seven most frequent encountered PCR ribotypes. Data from the sentinel surveillance and from the ad hoc typing are displayed separately. Period: May 1st 2012 – May 1st 2013. The category 'other types' consists of 59 different types in the Sentinel surveillance data and 34 different PCR-ribotypes in the ad hoc typing data.





Conclusions and recommendations

- The Reference Laboratory is now capable to recognize 164 different PCR ribotypes, including two new ribotypes (Type 237 and 244). This year, 83% of the samples submitted for ad hoc typing and 89% of the samples submitted by participants of the sentinel surveillance contained *Clostridium difficile.*
- The results of the sentinel surveillance in 19 hospitals revealed that the mean incidence was 14.7 per 10,000 hospital admissions (varying from 5 to 27 per 10,000 admissions). This incidence is similar to the incidence of 15 per 10,000 admissions that was reported in the recent years.
- The most frequent encountered PCR ribotypes included Type 014 (16%), Type 001 (14%), and Type 078 (12%).Twenty-eight isolates were characterized as Type 027 (3%) and no type 027-like ribotypes (016, 036, 176) were found. The closely related ribotypes 078 and 126 were found in 15% of the isolates. Type 078 seems to be associated with an higher rate of complicated courses compared to other ribotypes excluding Type 027. However, further research is needed to confirm this finding. Literature have showed that strain 078 (containing the virulence factors TcdA, TcdB, binary toxin and deletion in tcdC) caused more severe CDI and an higher mortality compared to other strains excluding Type 027, although this was not found in all studies.[2] [23]
- A total of 150 patients (25%) had severe CDI. After 30 days, six patients were admitted to the ICU as a consequence of CDI and two underwent a colectomy; 56 patients with CDI (11%) died. Twelve deaths were contributable to CDI.
- It is estimated that more than 2900 hospitalized patients annually will develop CDI of which 100 will succumb attributable or 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 2011 and May 1st 2012, one large outbreak caused by Type 027 and two small outbreaks (Type 001, and Type 015) were observed in participating hospitals to the sentinel surveillance. Type 027 was sporadically found in five participating hospitals, but did not cause any outbreak.
- In the period between May 1st 2012 and May 1st 2013, 296 samples from 17 healthcare facilities were send to the Reference Laboratory for typing, because severe clinical forms of CDI were observed or outbreaks suspected. PCR ribotype 027 was more frequently found (20%) compared to 2011-2012 (15%). This is attributable to a large persisting outbreak since January 2011, that encompassed at least 69 isolates in a hospital in the eastern part of the Netherlands and surrounding nursing homes.
- We also found isolates that were related to this particular strain in three hospitals using MLVA.
- Two outbreaks due to Type 027 involved both nursing home residents and hospitalised patients. There is no systematic surveillance for CDI in nursing homes yet, but similar as in 2012, we recommend strongly to initiate this.

[23] Walker AS et al, Relationship Between Bacterial Strain Type, Host Biomarkers, and Mortality in Clostridium difficile Infection, Clinical Infectious Diseases 2013;56(11):1589–600.
[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

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; Point Prevalence Study: European multi-centre prospective bi-annual point prevalence study of the incidence of Clostridium difficile Infection in patients with nosocomial diarrhoea (EUCLID).

Publications May 2012 – May 2013 related to the reference laboratory

Hensgens MP, Goorhuis A, Dekkers OM, Kuijper EJ. Time interval of increased risk for *Clostridium difficile* infection after exposure to antibiotics. J Antimicrob Chemother. 2012;67:742-8

Arvand M, Moser V, Schwehn C, Bettge-Weller G, Hensgens MP, Kuijper EJ. High prevalence of *Clostridium difficile* colonization among nursing home residents in Hesse, Germany. PLoS One. 2012;7(1):e30183.

Hensgens MP, Keessen EC, Squire MM, Riley TV, Koene MG, de Boer E, Lipman LJ, Kuijper EJ; on behalf of European Society of Clinical Microbiology and Infectious Diseases Study Group for Clostridium difficile (ESGCD). *Clostridium difficile* infection in the community: a zoonotic disease? Clin Microbiol Infect. 2012l;18:635-645.

Burt SA, Siemeling L, Kuijper EJ, Lipman LJ. Vermin on pig farms are vectors for *Clostridium difficile* PCR ribotypes 078 and 045. Vet Microbiol. 2012 May 18. [Epub ahead of print]

Knetsch CW, Terveer EM, Lauber C, Gorbalenya AE, Harmanus C, Kuijper EJ, Corver J, van Leeuwen HC. Comparative analysis of an expanded *Clostridium difficile* reference strain collection reveals genetic diversity and evolution through six lineages. Infect Genet Evol. 2012;12:1577-85

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 Apr 8;2(1)

van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JF, Tijssen JG, Speelman P, Dijkgraaf MG, Keller JJ. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. N Engl J Med. 2013;368:407-15.

Hensgens MP, Goorhuis A, Dekkers OM, van Benthem BH, Kuijper EJ. All-cause and disease-specific mortality in hospitalized patients with *Clostridium difficile* infection: a multicenter cohort study. Clin Infect Dis. 2013;56:1108-16.

Kim J, Kang JO, Kim H, Seo MR, Choi TY, Pai H, Kuijper EJ, Sanders I, Fawley W. Epidemiology of *Clostridium difficile* infections in a tertiary-care hospital in Korea. Clin Microbiol Infect. 2013 Jun;19(6):521-7. doi: 10.1111/j.1469-0691.2012.03910

Arvand M, Moser V, Schwehn C, Bettge-Weller G, Hensgens MP, Kuijper EJ. High prevalence of *Clostridium difficile* colonization among nursing home residents in Hesse, Germany. PLoS One. 2012;7(1):e30183. doi: 10.1371.

Reil M, Hensgens MP, Kuijper EJ, Jakobiak T, Gruber H, Kist M, Borgmann S. Seasonality of *Clostridium difficile* infections in Southern Germany. Epidemiol Infect. 2012;140:1787-93.

Koene MG, Mevius D, Wagenaar JA, Harmanus C, Hensgens MP, Meetsma AM, Putirulan FF, van Bergen MA, Kuijper EJ. *Clostridium difficile* in Dutch animals: their presence, characteristics and similarities with human isolates. Clin Microbiol Infect. 2012;18:778-8.

E.J. Kuijper and A. Haenen. *Clostridium difficile* bij ouderen in ziekenhuis en verpleeghuis. Themanummer Infectieziektebestrijding in verzorgingshuizen en verpleeghuizen, Tijdschrift voor Ouderengeneeskunde. 2013; 38 no. 1:32-3.

Presentations and posters May 2012 – May 2013

NVMM, voorjaarsvergadering 16 en 17 April, 2013, Papendal.

(oral) E.C. Keessen, C. Harmanus, M.E.H. Bos, W.E. Dohmen, D.J.Heederik, J.A Wagenaar, E.J. Kuijper, L.J.A Lipman. *Clostridium difficile* 078 in pigs: a threat for farmers and employees.

(oral) C.W. Knetsch, L. Keessen, M. He, L. Lipman, E.J.Kuijper,m J. Cover, T.D. Lawley. Whole genome sequencing reveals potential transmission of *C. difficile* Type 078.

(oral). S.M. van Dorp, E.J. Kuijper, E.A Verspui, W.C van Zwet, I. Frenay, D.W Notermans. Outbreaks of *Clostridium difficile* Type 027 infections in nursing homes: tip of the iceberg?

(poster). I.M.J.G Sanders, W. Knetsch, E. Claas, M. Wilcox, E.J. Kuijper, J.Corver. Novel multiplex RT-PCR for the detection of lineage specific *C. difficile* strains.

22nd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), 27-30 April 2013, Berlin.

P2502. Emergence of *Clostridium difficile* Type 176 in Prague University Hospital
M. Krutova*, O. Nyc, E.J. Kuijper, C. Harmanus, J. Matejkova, H. Nekvasilova, M. Solarova (Prague, CZ; Leiden, NL)

P2494. Emergence of *Clostridium difficile* polymerase chain reaction (PCR)-ribotype 027 in Hesse, Germany. M. Arvand*, V. Moser, B. Scholl, S. Winter, G. Bettge-Weller, C. Harmanus, E.J. Kuijper (Dillenburg, DE; Leiden, NL

P2495. First results of a Polish surveillance programme of *Clostridium difficile* infections: high prevalence of *Clostridium difficile* polymerase chain reaction (PCR) ribotype 027
H. Pituch*, P. Obuch-Woszczatynski, D. Lachowicz, G. Mlynarczyk, D. Wultanska, E.J. Kuijper (Warsaw, PL; Leiden, NL)

P2496. Emergence of *Clostridium difficile* infection in tuberculosis patients due to a highly rifampicin-resistant clone, polymerase chain reaction ribotype 046 in Poland. P. Obuch Woszczatynski, G. Dubiel, C. Harmanus, E.J. Kuijper, U. Duda, D. Wultanska, H. Pituch* (Warsaw, Bystra Slaska, PL; Leiden, NL; Bielsko Biala, PL)

O444. *Clostridium difficile* infections in general practice in the Netherlands. M.P.M. Hensgens*, A. Demeulemeester, O.M. Dekkers, A. Buiting, P. Bloembergen, S. Le Cessie, E.J. Kuijper (Leiden, Breda, Tilburg, Zwolle, NL)

O452. Antimicrobial activity of LFF571 and three treatment agents against *Clostridium difficile* isolates collected at a pan-European survey. S.B. Debast*, M.P. Bauer, I.J.M.G. Sanders, M.H. Wilcox, E.J. Kuijper for the ECDIS Study Group

P1869. Diagnostic testing and measurement of *Clostridium difficile* infections across Europe. S. van Dorp*, M.P.M. Hensgens, A. Virolainen, E. Nagy, P. Mastrantonio, K. Ivanova, F. Fitzpatrick, F. Barbut, V. Hall, T. Eckmanns, C. Suetens, K.A. Davies, M.H. Wilcox, D. Notermans, E.J. Kuijper on behalf of the ECDIS-Net participants

Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC 2012) September 9-12 San Francisco, California.

Marjolein P.M. Hensgens, A. Demeulemeester, Olaf M. Dekkers, A. Buiting, P. Bloembergen, Birgit H.B. van Benthem, Ed J. Kuijper. Case-Control Study of Community-onset *Clostridium difficile* Infections in The Netherlands. Poster, ICAAAC 2012, San Francisco.

Marjolein P.M. Hensgens, Abraham Goorhuis, Olaf M. Dekkers, Birgit B.H. van Benthem, Ed J. Kuijper. Mortality of Nosocomial *Clostridium difficile* Infections; a Multicenter Cohort study. Oral, ICAAC 2012, San Francisco

4th International *Clostridium difficile* symposium; September 20-22, 2012 Bled, Slovenia

Ed J, Kuijper. Oral, INV9; Diagnostics and typing of Clostridium difficile infections in Europe.

Ed J Kuijper and E.C Keesssen, Oral; 026. *Clostridium difficile* 078 in pigs: a threat for farmers, their relatives and employees.

K Rosenbusch, D Bakker, EJ Kuijper and WK Smits. DNA binding by the master regulator of sporulation, *Spo0A*, of *Clostridium difficile.* Poster P49

Hans C. van Leeuwen, Ed. J. Kuijper and J. Corver. *Clostridium difficile* TcdC binds quadruplex DNA structures. Poster P56

D. Elina, I Ivanov, R. Dobrevska, K. Ivanova, M. Marina. P. Petrov, T. Kantardjiev, EJ Kuijper. Implementation of molecular methods for identification, detection of toxin encoding genes and typing in *Clostridium difficile*. Poster, P86.

W Knetsch, I Sanders, E. Claas, M. Wilcox, E Kuijper and J Corver. Novel multiplex RT-PCR for the detection of lineage specific *Clostridium difficle* strains. Poster, P89.

J. Blanco. S. Alvarez, T. Pelaez, M. Lanzarot, B Gama, C. Harmanus, EJ Kuijper, E. Bouza and ME Garcia. Isolation of *Clostridium difficile* from puppies: a longitudinal study. Poster, P107.

J. Blanco. S. Alvarez, T. Pelaez, R. Astorga, B Gama, C. Harmanus, EJ Kuijper, E. Bouza and ME Garci. High recovery rate of *Clostridium difficile* PCR ribotype 078 in Iberian free-range pigs. Poster, P108.

E. van Eijk, AH. Friggen, P. Soultanas, WK. Smits. Identification and characterization of the replication machinery of *Clostridium difficile*, Poster, P51

Invited presentations May 2012 – May 2013

21 May 2012, Leiden University Medical Centre; visit Delegation of China Ministry of Health & Expert Committee for Rational Drug Use to LUMC . Dr. Cheng Ying, Chinese Center of Disease Control, and dr. Ed J. Kuijper, LUMC. Antibiotic associated disease due to *Clostridium difficile* in China and Europe

30 August-2 September 2012 NSCMID, 2012 (Annual meeting of Nordic Society of Clinical Microbiology and Infectious Diseases). Ed J. Kuijper and D Notermans; oral presentation; Epidemiology and diagnosis of CDI.

Defense thesis Copenhagen. CDI in Denmark: Epidemiology, risk factors and clinical presentation. Lilian Marie Soes, 5 October 2012

Health Protection 2012, Warwick University on 11-12 September. Oral presentation. Ed J Kuijper and DW Notermans. A global perspective of *Clostridium difficile* epidemiology

Nederlandse Vereniging voor bioMedisch Laboratoriummedewerkers. *Clostridium difficile* en VRE laten hun sporen na'. 5 (Leeuwarden) en 12 juni (Rotterdam) 2012.

Hospital Infection Society International Conference 19-21 th November -2012. Oral Presentation. Ed J Kuijper; Faecal transplantation and other approaches for patients with recurrent *Clostridium difficile* infection

14 November, 2012 Symposium fur Milkrobiologische Diagnostik, Luzern, Swiss. Oral presentation: "CDI; changing epidemiology and new treatment options."

Marjolein P.M. Hensgens, Birgit H.B. van Benthem, Daan W. Notermans en Ed J. Kuijper . Resultaten van de Nederlandse nationale surveillance voor *Clostridium difficile* infecties. VHIG 2012, oral presentation, Almelo

European Veterinary Conference, 18-20 April 2012, Amsterdam. Liny Keessen, dr. Len Lipman, dr. Daan Notermans en prof. dr. Ed Kuijper. *Clostridium difficile* bij dieren en zoönotische aspecten

27 May, 2012. Frankfurt. *Clostridum difficile,* Aktuelles zur Epidemiologie, Diagnostik und Therapie. Ed J Kuijper, M. Hensgens, S. van Dorp and D. W. Notermans: CDI outside the hospitals: community-associated and zoonotical acquired.

Organization of Workshops and congress sessions.

Participation of prof. Wilcox and prof. Kuijper in HPA/NHS organized workshop: CLOSTRIDIUM DIFFICILE THE NEXT FRONTIER: EDUCATION AND LEARNING EVENT FOR PROFESSIONALS IN HEALTH AND SOCIAL CARE . Tuesday 4th December 2012 Parade Ring Room, Ascot Racecourse, Ascot, Berkshire, SL5 7JX

NVMM voorjaarsvergadering, 16 en 17 April 2013. Community acquired *Clostridium difficile* infections: from pigs to humans and vice versa.

Educational workshop ECCMID 2013, Berlin: "*Clostridium difficile* in 2013: what's new?".