Sixth Annual Report of the National Reference Laboratory for *Clostridium difficile* (May 2011 to May 2012) and results of the sentinel surveillance

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Content

Abstract **3**
Introduction **4**
Aims and procedures of the sentinel surveillance **5**
Aims and procedures of the ad hoc typing **6**
Results of the sentinel surveillance **7**
Participating hospitals 7
Outbreaks in participating hospitals 7
Incidence in participating hospitals 8
Demographical and clinical data of the sentinel surveillance 8
Table 1 8
Figure 1 9
Table 2 10
Table 3 11
Results of the ad hoc typing **12**
Hospitals using the Reference Laboratory 12
Strains sent to the Reference Laboratory 12
Figure 2 13
Figure 3 14
Table 4 15
Figure 4 16
Conclusions and recommendations **17**
Output **18**
Participation of National Reference Laboratory in National and European activities 18
Publications June 2011 – June 2012 18
Presentations and posters June 2011 – June 2012 19
Organization of Workshops 20

Abstract

As of 2005, outbreaks with *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). 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. Surveillance resulted in recognition of new *C. difficile* PCR ribotypes, such as type 078 which was also found increasingly in other European countries. As of May 2009, **sentinel surveillance was started**, with PCR ribotyping performed by the LUMC.

The **results of 18 hospitals participating in the sentinel surveillance** revealed that the mean incidence of CDI is 15 per 10.000 admissions, varying from 3 to 29 per 10.000 admissions. The incidence rates were not influenced by implementation of molecular tests to diagnose CDI, introduced in 2 laboratories. Among the 835 isolates cultured, type 001 was the most frequently found type (17%), type 014 was found in 13% and type 078 in 12%. Type 027 was found in 3% in seven hospitals. A total of 157 patients (27%) had severe CDI. After 30 days, 1 patient was admitted to the ICU as a consequence of CDI and 3 underwent a colectomy; 62 patients with CDI (13%) died. Eighteen deaths were contributable to CDI.

Extrapolating the data of sentinel surveillance to all hospitals in the Netherlands, it is estimated that **more than 2700 hospitalized patients annually** will develop CDI of which 100 will succumb attributable or contributable to 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

Between May 1st 2011 and May 1st 2012 **twelve outbreaks** were seen in the sentinel surveillance and **two** in the ad hoc typing.

In the period between May 1st 2011 and May 1st 2012, 289 samples from 26 healthcare facilities and laboratories in the Netherlands were investigated in **the ad hoc typing**. Type 001 and type 027 were the most commonly found PCR ribotypes (both in 15.0%), followed by type 078 (12.2%) and type 014 (11.4%). **Type 027 was less frequently found compared to last year (26%), but still very common.** Most of type 027 infections were found in one hospital with a large outbreak and a hospital with numerous type 027, this year not meeting the criteria of an outbreak. The largest outbreak due to type 027 took place in an elderly home.

The Reference Laboratory is now able to recognize and name 158 types. Additionally, 130 different unknown types were differentiated by the Reference Laboratory between 2005 and 2011. The Reference Laboratory is not yet able to name these isolates, therefore, 12 'unknown' types that occurred more than 5 times and were sent to the NHS Clostridium Reference Laboratory in Cardiff, Wales to name these isolates.

We conclude that type 027 is again a frequently found type in healthcare facilities that do not participate in the surveillance. This is due to two large outbreaks caused by this strain. Besides these outbreaks, the incidence seems stable, with types 001, 014 and 078 as the predominant types.

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 400 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, 158 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.

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 Reference Laboratory 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 one of the 158 PCR ribotypes that are known by the Reference Laboratory. Together with the submission of samples (feces 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.

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.[1] [2] [3] Furthermore, a decrease in type 027 was noted, together with an increase of the hypervirulent type 078.[14] This type has also been found as an increasing type in Europe and is currently the third most frequent found type Europeanwide.[5] Interestingly, *C. difficile* type 078 has also been recognized as the most important agents of piglet-associated diarrhea. [6]

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 third annual report that provides an overview of the two types of surveillance conducted in the Netherlands. The previous two 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. In this annual report we describe the situation in the Netherlands between May 1st 2011 and May 1st 2012.

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

[2] 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

[3] Goorhuis et al, Risk factors and outcome of *Clostridium difficile* infection due the four predominant PCR-ribotypes in The Netherlands, submitted

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

[5] 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.

[6] 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

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 consulted annual reports of the participating hospitals to note their number of admissions and number of admission-days. 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.[5] [6] 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 SPSS for Windows software package, version 17.0.
An outbreak was defined as >2 isolates of the same type, found less than 7 days apart in one hospital department.
A small outbreaks was defined as >2 and ≤4 isolates of the same type, found less than 7 days apart in one hospital department.

[5] Kuijper et al. Emergence of *Clostridium difficile*-associated disease in North America and Europe. Clin Microbiol Infect 2006; 12 Suppl 6:2-18
[6] 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 home made 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*.[7] All *C. difficile* strains are further investigated by PCR-ribotyping.[8] The presence of *tcdA*, *tcdB* and binary toxin genes is investigated according to standardized techniques.[9] [10] [11] Deletions in *tcd*C were determined by PCR using in-house designed primers.[12]

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 17.0. The definitions applied in this questionnaire are based on those proposed by the ECDC and the CDC.[13] [14]

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

[8] 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

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

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

[11] 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

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

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

[14] 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 started in May 2009. The sentinel surveillance was initiated to obtain systematic and representative incidence data and to identify new circulating ribotypes in the Netherlands. Therefore, we included both academic centers (n=5) and general hospitals (n=13), distributed equally in the Netherlands. This year, 18 hospitals actively joined the sentinel surveillance. Previous years 19 or 20 participating centers were participating, however, as was shown in the previous report, not all were actively contributing with data and isolates. In 2012, two new hospitals joined the surveillance. One hospital participated before, but was inactive for some months, the other is new in the surveillance and started participation because of an increased incidence of CDI. The geographical distribution of the participating centers is displayed in figure 1.

Between May 1st 2011 and May 1st 2012, the 18 participating centers sent in 941 samples. These 941 samples were all cultured and resulted in 835 (88.7%) *C. difficile* isolates. Type 001 was the most frequently found type, isolated in 138 of the 835 isolates (16.5%). Type 014 was found in 112 isolates (13.4%), type 078 in 102 isolates (12.2%) and type 002 in 48 isolates (5.8%). Twenty-six isolates were positive for type 027 (3.1%) and 75 isolates (9.0%) had a PCR ribotype which pattern was not recognized in our database. When type 078 is combined with PCR ribotype 126, which is closely related according to MLST[15], this combination was found in 14.5%. The results per participating center are displayed in Table 2.

Compared to the previous year 2010-2011, no major changes were noticed, although type 001 was less frequently found this year. Distribution of the various PCR ribotypes last year consisted of the following percentages: type 001 20%, type 014 13%, type 078 12%, type 002 6%. Twenty-four isolates were positive for type 027 (3%).

This year we added a column with applied diagnostic methods for CDI to the table. Three hospitals introduced the more sensitive PCR for toxin detection during the previous years (2010 or 2011), therefore we evaluated whether this influenced the incidence rates. The institutes who used a PCR were not associated with the highest incidence figures in the current report. Besides, the three hospitals did not show a consistent increase of CDI incidence per 10.000 admissions since introduction of PCR (9 → 17, 22 → 15 and 27 →21 per 10.000 admissions in 2009-2010 and 2011-2012, respectively).

Besides the 3 hospitals that used a PCR for the diagnosis of CDI during the total period of this annual report, 1 other hospital introduced a PCR for toxin detection last year and two hospitals introduced LAMP (loop-mediated isothermal amplification) for toxin detection. Next year we will evaluate whether this influenced incidence rates.

Outbreaks in participating hospitals

Between May 1st 2011 and May 1st 2012, two relatively large outbreaks and ten small outbreaks (up to 4 isolates involved) were observed in seven participating hospitals. A large outbreak was caused by type 001 (70 isolates of type 001 found). This outbreak was also present last year, with a similar number of patients involved (78 isolates between 2010-2011). Six isolates with an unknown type were found within 7 days apart in another hospital.

The ten small outbreaks (>2 and ≤4 isolates of the same type, found less than 7 days apart in one hospital department) were identified. Types causing these outbreaks were: 001, 002, 015, 078, 126 and an unknown type. Type 078 was responsible for three small outbreaks, type 001 for two.

In the previous year, type 001 was also frequently found in outbreaks. Type 014, 027 and 078 were known to cause outbreaks in the previous years.

Hospital O joined the sentinel surveillance this year, because they had more patients with CDI than in previous years. According to their participation during 3 months of 2012, their incidence was only somewhat higher (17 / 10.000 admissions) than the mean incidence. Type 027 was the most frequently found type (n=11). According to our definition of an outbreak (>2 isolates of the same type, found less than 7 days apart in one hospital department), there was no outbreak. However, the

[15] Stabler RA, et al. Macro and micro diversity of Clostridium difficile isolates from diverse sources and geographical locations. PLoS One. 2012;7(3):e31559.

relative frequent isolation of type 027 warrants close monitoring of the situation in the following months.

Incidence in participating hospitals

The incidence of CDI was measured using the data of the months in which patients were actively included. Fourteen hospitals (78%) participated actively during the whole study period (11 or 12 months of active participation). Among the four hospitals that did not participate actively, two hospitals started the surveillance in January 2012 after which they actively participated. The remaining two hospitals actively participated in the surveillance during 7 months.

Data about number of admissions and number of admission-days were known for all participating hospitals. The mean incidence was 15.4 / 10.000 hospital admissions (varying from 3 to 29 per 10.000 admissions). This incidence comparable to the incidence of 15 per 10.000 admissions that was reported last year and the incidence of 18 per 10.000 admissions that was reported in the Netherlands in 2005.[16] When the incidence of 2011-2012 was weighted by number of admissions and therefore, the larger hospitals are assigned a higher weight, the incidence was somewhat higher: 16.8 / 10.000 admissions.

Demographical and clinical data of the sentinel surveillance

Demographical and clinical characteristics were collected of all patients that were included in the sentinel surveillance. Of the 733 patients included, 347 were male (48.3%) and 372 were female (51.7%). The mean age was 66 years (SD 20.1), varying from 2 to 99 years. 221 (31.3%) patients had community-onset CDI, the remaining 486 patients (68.7%) were admitted in a healthcare center when the diarrhoea started. These patients were most frequently admitted to a hospital (n=430; 88.5%) at the department of internal medicine (n=166; 38.6%) and general surgery (n=60; 14.0%). Sixteen patients were admitted to the intensive care unit (3.7%).

Most patients used antibiotics prior to the start of diarrhoea (n=417; 72.5%). In the eight weeks prior to the start of this episode of diarrhoea, 61 patients (15.0%) had an episode of CDI.

A total of 157 patients (27.0%) had severe CDI, defined as bloody diarrhea and/or diarrhea with hypovolaemia 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 494 patients. 428 patients (86.6%) had an uncomplicated course of their CDI infection. After 30 days, 1 patient (0.2%) was admitted to the ICU as a consequence of CDI, 3 patients (0.6%) needed surgery as a consequence of their CDI and 62 patients with CDI (12.6%) died. Eighteen deaths (3.6%) were contributable to CDI. Three of these patients had CDI due to type 078 and another three patients had CDI due to type 014. Other types involved in contributable death were type 001, 002, 003, 046, 056, 126, and 159.

Table 1: Outcome of CDI patients, stratified per PCR ribotype

	PCR ribotype					
	027		078		other	
	N	%	N	%	N	%
Severe diarrhoea	3/11	27,3	18/50	36,0	98/379	25,9
Complicated course	3/11	27,3	10/49	20,4	35/311	11,3
Complicated course due to CDI	0/11	0,0	4/49	8,2	14/311	4,5

A complicated course is defined as: death <30 days
A complicated course due to CDI is defined as: death <30 days due to CDI, colectomy or ICU admission due to CDI

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

Figure 1: Participating hospitals of the sentinel surveillance

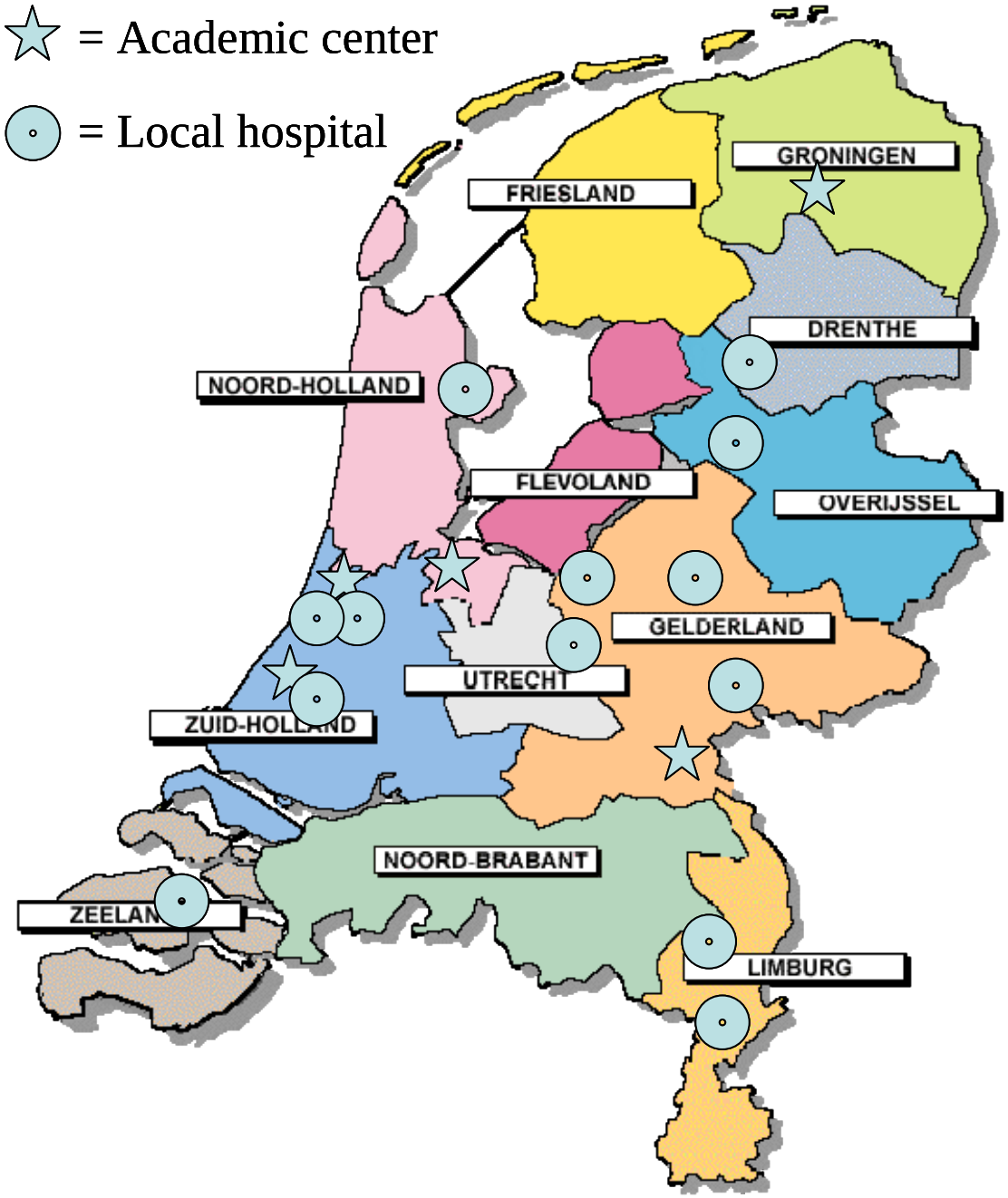


Table 2: Isolates submitted for PCR ribotyping by hospitals participating in the sentinel surveillance. Period: May 1st 2011 – May 1st 2012.

Participating hospital	Samples*		Faeces		*C. difficile*		Most common type			2nd most common type		
	(N)	(%)	(N)	(%)	(N)	(%)		(N)	(%)		(N)	(%)
A	130	13,8	4	3,1	119	91,5	001	70	58,8	078	8	6,7
B	52	5,5	0	0,0	45	86,5	014	8	17,8	078	6	13,3
C	87	9,2	4	4,6	81	93,1	001	17	21,0	014	12	14,8
D	43	4,6	0	0,0	41	95,3	001	7	17,1	014	7	17,1
E	51	5,4	49	96,1	35	68,6	014	8	22,9	001	4	11,4
F	9	1,0	0	0,0	8	88,9	014	2	25,0	023	2	25,0
G	54	5,7	0	0,0	54	100,0	078	13	24,1	002	7	13,0
H	37	3,9	1	2,7	33	89,2	001	9	27,3	078	5	15,2
I	11	1,2	1	9,1	8	72,7	001	2	25,0	005	2	25,0
J	59	6,3	0	0,0	36	61,0	014	8	22,2	078	6	16,7
K	1	0,1	0	0,0	1	100,0	078	1	100,0	-	-	-
L	94	10,0	0	0,0	94	100,0	014	18	19,1	078	12	12,8
M	16	1,7	0	0,0	14	87,5	014	4	28,6	unknown	4	28,6
N	54	5,7	53	98,1	46	85,2	027	8	17,4	017	6	13,0
O	36	3,8	35	97,2	32	88,9	027	11	34,4	002	4	12,5
P	56	6,0	52	92,9	43	76,8	014	10	23,3	005	5	11,6
Q	11	1,2	6	54,5	9	81,8	078	2	22,2	010	1	11,1
R	140	14,9	0	0,0	136	97,1	078	19	14,0	unknown	19	14,0
Total	**941**	**100**	**205**	**21,8**	**835**	**88,7**	**001**	**138**	**16,5**	**014**	**112**	**13,4**

* Isolates and faecal samples

Table 3: Number of patients included in the sentinel surveillance per location and incidence data. Period: May 1st 2011 – May 1st 2012. The incidence data were calculated using only the months of active participation. The type of screening test used for toxin detection is shown per hospital.

Participating hospital	Months of active participation	Patients (N)	Patients (%)	Monthly admissions	Monthly admission-days	Incidence / 10,000 admissions	Incidence / 10,000 admission-days	Diagnostic test
A	11	87	12%	2756	12953	28,70	6,11	EIA* or LAMP (Mar '12)
B	7	40	5%	2511	16880	22,76	3,39	EIA
C	12	87	12%	3081	14524	23,53	4,99	EIA^
D	12	43	6%	2472	11008	14,50	3,26	PCR
E	12	47	6%	2964	15414	13,21	2,54	EIA*
F	12	20	3%	1952	8736	8,54	1,91	EIA*
G	7	30	4%	2987	26788	14,35	1,60	EIA*
H	3	7	1%	1583	7204	14,74	3,24	EIA* or LAMP (Oct '11)
I	12	20	3%	1553	7583	10,73	2,20	EIA^
J	12	22	3%	1786	10938	10,27	1,68	EIA^
K	12	4	1%	1042	5327	3,20	0,63	EIA^
L	12	76	10%	3059	15266	20,70	4,15	PCR
M	12	21	3%	1213	7544	14,43	2,32	EIA
N	12	45	6%	3481	24067	10,77	1,56	EIA^
O	3	11	2%	2123	10021	17,27	3,66	EIA*
P	12	54	7%	2580	15992	17,44	2,81	PCR
Q	12	8	1%	866	4246	7,69	1,57	EIA*
R	12	111	15%	3952	19077	23,40	4,85	EIA* or PCR (Jan '12)
		733	**100%**	**41961**	**233568**	**15,35**	**3,71**	

LAMP = Loop-Mediated Isothermal Amplification
EIA = enzyme immunoassay
* = Immunocard toxin A and B (Meridian)
^ = Vidas Clostridium toxin A and B (Biomerieux)

Results of the ad hoc typing

Hospitals using the Reference Laboratory

In the period between May 1st 2011 and May 1st 2012, 26 healthcare facilities and laboratories in the Netherlands sent samples to the Reference Laboratory in Leiden, excluding healthcare facilities participating in the sentinel surveillance. In total, 289 samples were submitted of which 6.2% consisted of feces samples. Fecal samples were submitted by 10 of the 26 facilities. Of these 10 facilities, 2 were capable of submitting strains, the other eight facilities submitted only fecal samples.

Strains sent to the Reference Laboratory

Of the 289 samples submitted, 85.1% was positive for *Clostridium difficile* in culture. This was higher than the results of the Reference Laboratory in 2010-2011 when 76% of the samples submitted contained *C. difficile*, but equal to previous years. Type 001 and type 027 were the most commonly found PCR ribotypes (both in 15.0%), followed by type 078 (12.2%) and type 014 (11.4%); see table 4 and figure 4. The percentage of type 027 decreased compared to the previous year: 25.5% in 2010-2011, but was higher than found in 2009-2010 (4.3%). Between 2005 and 2007 the incidence of type 027 was 12% in 2006-2007 and 21% in 2005-2006. Besides type 027, type 001, 014 and 078 were the three most frequently found PCR ribotypes. In the previous years, these types were also frequently found.[17]

Since 1st of May 2011, outbreaks due to two different types were observed. An outbreak was defined as >2 identical PCR ribotypes with a maximum interval of 7 days in one hospital.

Type 027 caused problems in two facilities (facility 1 and 3). Both experienced outbreaks as of May 2010 and January 2011, respectively. Between 2011 and 2012, we again observed an outbreak in facility 1 and typed several 027 strains in facility 3 (but the definition of an outbreak was not fulfilled). Between May 1st 2011 and May 1st 2012, 20 patients with type 027 were identified in facility 1 and 12 in facility 3. In total, type 027 was isolated 59 times during the outbreak in facility 1 and 29 times in facility 3. In Figure 2 we displayed the time span of both outbreaks. Type 027 was also sporadically found in 4 facilities (n=3, n=2, n=2 and n=8). A single isolate of type 027 was found in 5 other hospitals. In none of these hospitals an outbreak was detected.

Type 078 was responsible for one small outbreak (3 isolates involved). No other outbreaks were observed. In the previous report, in total 5 outbreaks were observed due to three different PCR ribotypes (n=71 type 027, n= 25 type 001 and n=3 type 014).

46 Type 027 isolates from 5 different hospitals isolated in 2011 and 2012 were further analysed by multilocus variable number of tandem repeats analysis (MLVA); see figure 3. Almost all the type 027 *C. difficile* strains from the different hospitals in the Netherlands between 2011 and May 2012 were related to each other. Within hospital A multiple identical strains (multiple isolates in the same circle) were found, which matches the epidemiological conclusion that there was an outbreak.

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

Figure 2: Outbreaks with type 027 in facility 1 and 3, stratified by month.

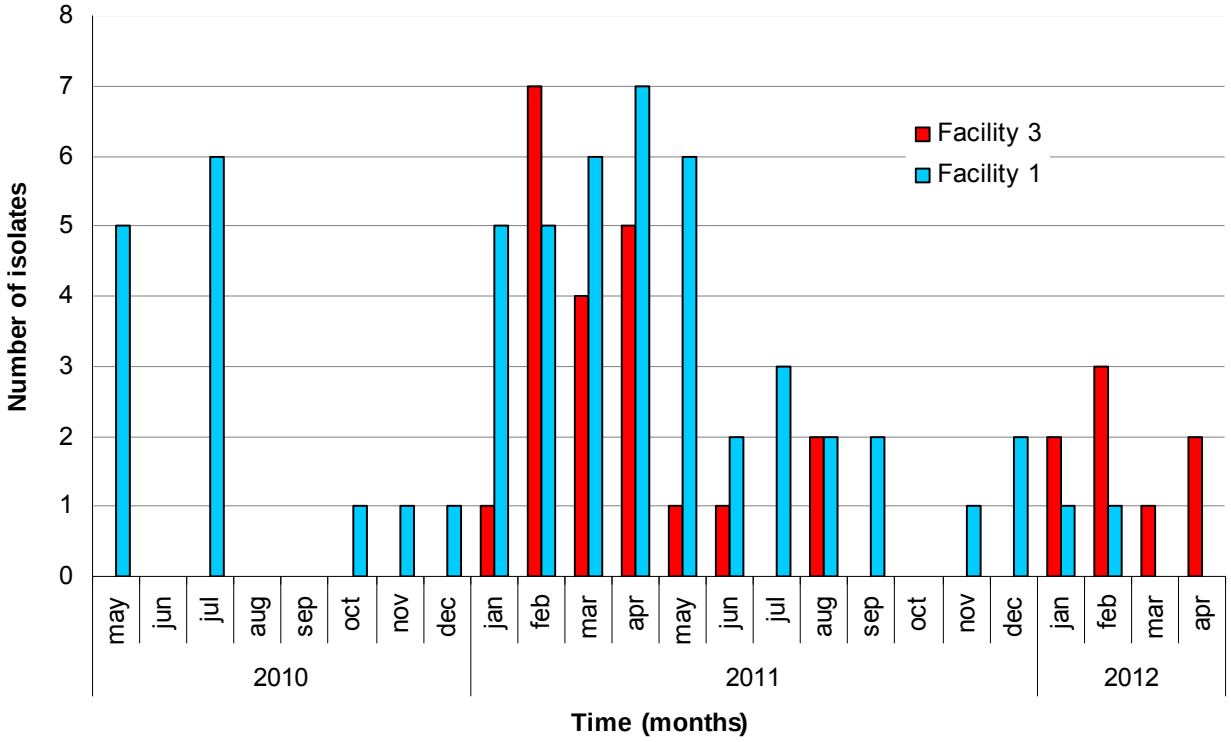


Figure 3: Minimal Spanning Tree of 46 PCR ribotype 027 isolates from different hospitals in the Netherlands, typed in 2011 and 2012 by multiple-locus variable-number tandem-repeat analysis (MLVA).

Note: Isolates are labeled by sample number; Hospital A – E (HA-HE) with the number of isolates tested from each hospital and the year they were tested. Facility 1 is similar to hospital B, facility 3 is hospital A. All isolates in the same circle are 100% identical. The different colours of the circles signify the different Hospitals tested. The different lines between the circles are the number of loci the isolates differ. The numbers on those lines represent the summed tandem repeat differences (STRDs) between MLVA types. All isolates in the grey cluster represent a large genetically related complex, defined by an STRD ≤ 10. Within the grey cluster, a clonal complex is defined by an STRD ≤ 2. All the isolates form a clonal complex, with the exception of eleven isolates (HA14-'11; HA22-'12; HA24-'11; HB4-'11; HB5-'11; HB7-'11; HC1-'11; HC2-'11; HD5-'12; HD6-'11 & HE1-'11). All the isolates form one large genetically related complex, with the exception of HA14-'11; HD5-'12 & HE1-'11, which are not clonal or genetically related to the other isolates.

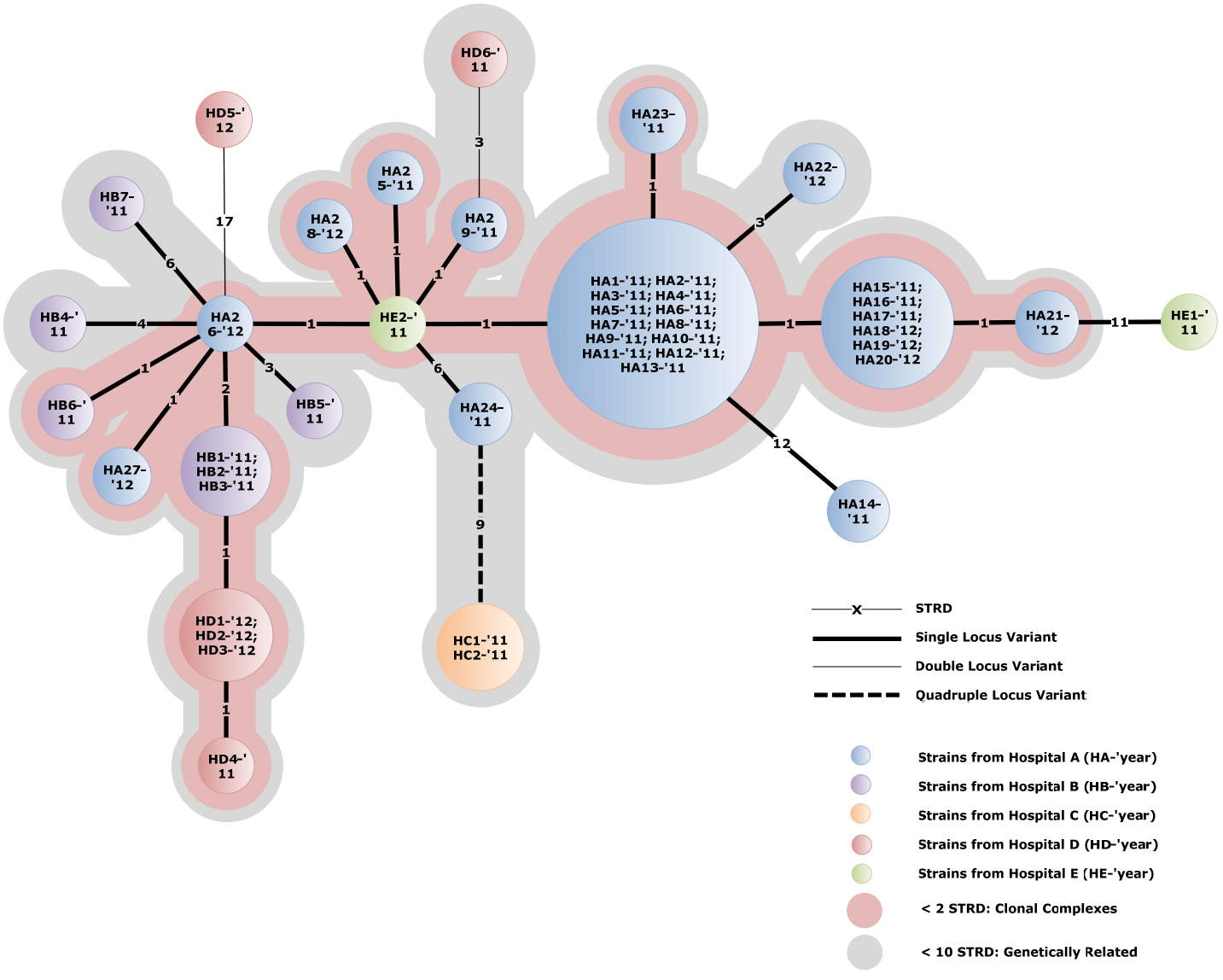
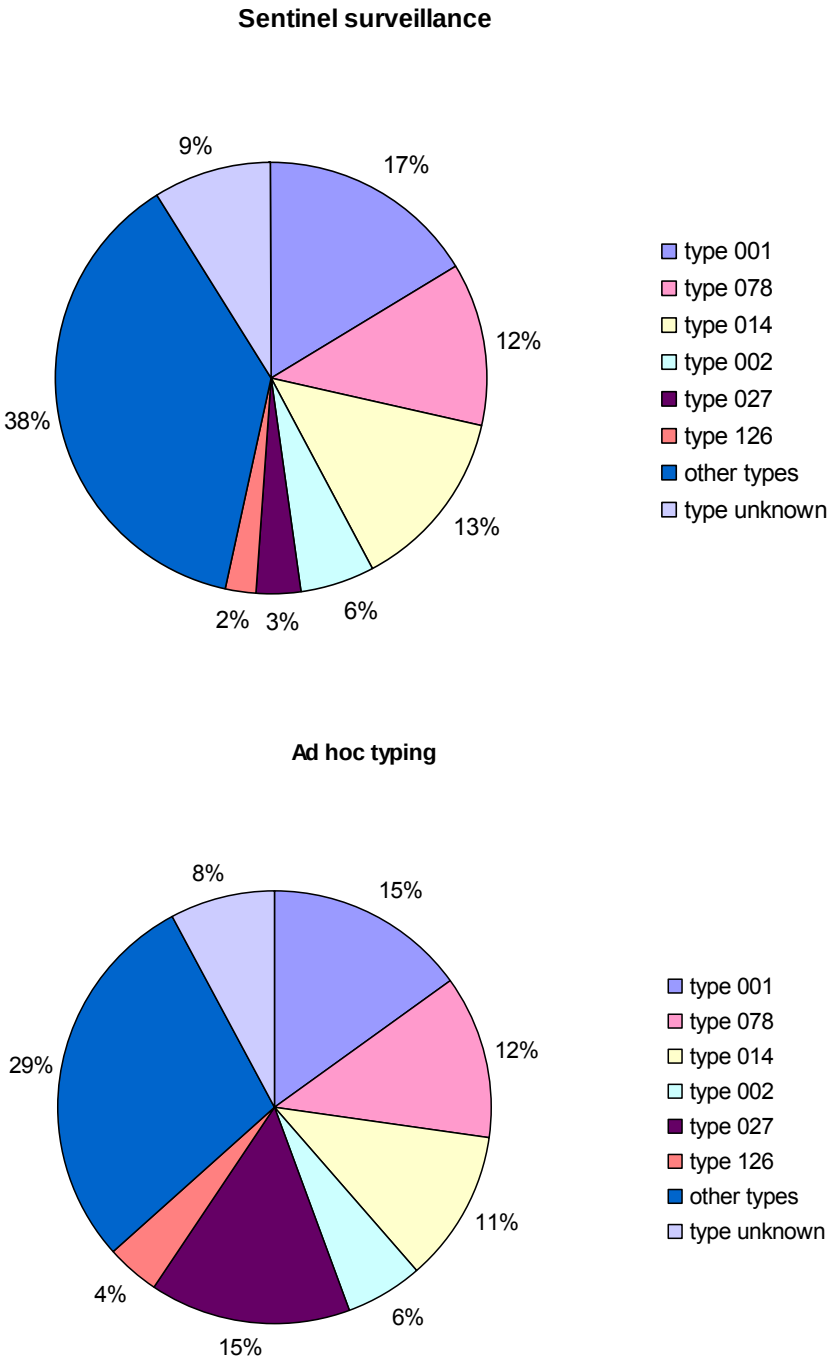


Table 4: Number of isolates sent to the Reference Laboratory for ad hoc typing per location. Period: May 1st 2011 – May 1st 2012. Hospitals that participate in the sentinel surveillance are not included.

	Samples*		Faeces		*C. difficile*		Most common type			2nd most common type		
Location	**(N)**	**(%)**	**(N)**	**(%)**	**(N)**	**(%)**		**(N)**	**(%)**		**(N)**	**(%)**
1	95	28,8	0	0	83	87	027	20	24	014	13	16
2	61	18,5	0	0	51	84	001	13	25	014	6	12
3	35	10,6	0	0	33	94	027	12	36	078	5	15
4	23	7,0	0	0	19	83	001	10	53	014	4	21
5	15	4,5	0	0	14	93	005	4	29	onbekend	3	21
6	8	2,4	0	0	8	100	027	3	38	onbekend	3	38
7	7	2,1	0	0	7	100	078	2	29	onbekend	2	29
8	7	2,1	0	0	7	100	078	3	43	001	1	14
9	6	1,8	0	0	2	33	001	1	50	014	1	50
10	4	1,2	4	100	4	100	050	2	50	078	2	50
11	4	1,2	0	0	4	100	001	4	100	-	-	-
12	3	0,9	0	0	1	33	087	1	100	-	-	-
13	3	0,9	3	100	0	0	-	-	-	-	-	-
14	3	0,9	2	67	2	67	014	1	50	070	1	50
15	2	0,6	2	100	0	0	-	-	-	-	-	-
16	2	0,6	1	50	2	100	027	1	50	078	1	50
17	2	0,6	2	100	2	100	012	1	50	078	1	50
18	1	0,3	1	100	0	0	-	-	-	-	-	-
19	1	0,3	0	0	1	100	126	1	100	-	-	-
20	1	0,3	0	0	1	100	078	1	100	-	-	-
21	1	0,3	1	100	0	0	-	-	-	-	-	-
22	1	0,3	0	0	1	100	001	1	100	-	-	-
23	1	0,3	0	0	1	100	005	1	100	-	-	-
24	1	0,3	1	100	1	100	001	1	100	-	-	-
25	1	0,3	0	0	1	100	106	1	100	-	-	-
26	1	0,3	1	100	1	100	005	1	100	-	-	-
Total	**289**	**100**	**18**	**6,2**	**246**	**85,1**	**001**	**41**	**16,7**	**027**	**37**	**15,0**

* Isolates and faecal samples

Figure 4: Percentage of 7 frequently encountered PCR ribotypes. Data from the sentinel surveillance and from the ad hoc typing are displayed apart. Period: May 1st 2011 – May 1st 2012. The category 'other types' consists of 36 different PCR-ribotypes in the ad hoc typing data and 62 different types in the sentinel surveillance data.



Conclusions and recommendations

- The Reference laboratory is now capable to recognize 158 different PCR ribotypes (134 last year).
- In the period between May 1st 2010 and May 1st 2011, 289 samples from 26 healthcare facilities and laboratories in the Netherlands were investigated at the Reference Laboratory in Leiden. These samples were submitted because of severe CID or an outbreak. Just like last year, type 027 was a frequently found PCR ribotype (15%). This was mainly due to two (former) outbreaks.
- The largest outbreak due to type 027 (encompassing now 59 isolates) took place in an elderly home. Because this outbreak is long lasting and outbreaks in elderly homes seem hard to combat, we started a prospective survey to test patients in nursing homes regularly using a specific algorithm. A pilot survey is underway and will be expanded to other nursing homes in 2012. We are also planning a retrospective study in nursing homes to analyse the severity and outcome of CDI in this elderly population.
- The increasing number of CDI cases from nursing homes deserves further attention and needs the introduction of specific algorithms and appropriate diagnostic facilities in the Netherlands.
- This year, 85% of the samples submitted for ad hoc typing were positive for *Clostridium difficile* in culture. In the previous annual report, we mentioned that this rate was lower (76%), and we subsequently decided to include a quality control by a second technician on negative tested samples. This resulted in a difference of 9.2% and therefore positive isolation rates increased. Next year we will continue reanalyzing all negative samples.
- The results of the sentinel surveillance in 18 hospitals revealed that the mean incidence of CDI is 15 per 10.000 admissions, varying from 3 to 29 per 10.000 admissions. Type 001 was the most frequently found type (17%), type 014 was found in 13% and type 078 in 12%. Type 027 was found in 3%. A total of 157 patients (27%) had severe CDI. After 30 days, 1 patient was admitted to the ICU as a consequence of CDI and 3 underwent a colectomy; 62 patients with CDI (13%) died. Eighteen deaths were contributable to CDI.
- During the period of sentinel surveillance, twelve outbreaks were observed due to types 001, 002, 015, 078, 126 and an unknown type. Type 027 did not cause outbreaks in the sentinel surveillance hospitals.
- The incidence of CDI in the Netherlands is unchanged compared previous years. Though *C. difficile* type 027 is re-emerging, this is restricted to a few hospitals. *C. difficile* type 078 has a stabile position and is the third most frequently found in the Netherlands, similar as other European countries. It is very likely that emerging CDI due to type 078 is related to the finding of *C. difficile* type 078 is pig farms, not only in the Netherlands, but also in Spain, England and Denmark. However, the small outbreaks with type 078 that occurred this year, mainly concerned patients who developed diarrhoea in the hospital (not in the community).
- It is estimated that more than 2700 hospitalized patients annually will develop CDI of which 100 will succumb attributable or contributable to CDI (with 114 hospitals and 1.845.000 hospital admissions per year, according to data from the Dutch Centraal Bureau voor de Statistiek)[18]. 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.

[18] http://statline.cbs.nl/StatWeb/publication/?VW=T&DM=SLNL&PA=71584NED&D1=0-1,8,24,27,30-41,55,57,70,83,95-130&D2=a&D3=l&HD=080528-1522&HDR=G2,G1&STB=T

Output

Participation of National Reference Laboratory in National and European activities

ECDC tender 2010-2013: Supporting capacity building for surveillance of *Clostridium difficile* infections at European level. Tenderer: Ed Kuijper

FP 7 Health; 2008-2011. The Physiological Basis of Hypervirulence in *Clostridium difficile*: a Prerequisite for Effective Infection Control. PI Nigel Minton (Nottingham).

ZonMW 2009-2013 (50-50800-98-079); Application of molecular virulence markers for rapid and improved diagnosis of *Clostridium difficile* infection due to hypervirulent strains. PI: Ed J. Kuijper

ZonMW 2009-2013 (50-50800-98-075); Reduction of community health risks of animal-associated *Clostridium difficile*. PI Len Lipman, Ed J. Kuijper

Publications June 2011 – June 2012

Hopman NE, Sanders IM, Kuijper EJ, Lipman LJ. Low risk of transmission of *Clostridium difficile* to humans at petting farms. Vet Microbiol. 2011;150:416-7

Reil M, Erhard M, Kuijper EJ, Kist M, Zaiss H, Witte W, Gruber H, Borgmann S. Recognition of *Clostridium difficile* PCR-ribotypes 001, 027 and 126/078 using an extended MALDI-TOF MS system. Eur J Clin Microbiol Infect Dis. 2011;30:1431-6.

Keessen EC, van den Berkt AJ, Haasjes NH, Hermanus C, Kuijper EJ, Lipman LJ. The relation between farm specific factors and prevalence of *Clostridium difficile* in slaughter pigs. Vet Microbiol. 2011;154:130-4.

Knetsch CW, Hensgens MP, Harmanus C, van der Bijl MW, Savelkoul PH, Kuijper EJ, Corver J, van Leeuwen HC. Genetic markers for *Clostridium difficile* lineages linked to hypervirulence. Microbiology. 2011;157:3113-23

Goorhuis A, Debast SB, Dutilh JC, van Kinschot CM, Harmanus C, Cannegieter SC, Hagen EC, Kuijper EJ. Type-specific risk factors and outcome in an outbreak with 2 different *Clostridium difficile* types simultaneously in 1 hospital. Clin Infect Dis. 2011;53:860-9

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. 2011 Aug 25. doi: 10.1111/j

Keessen EC, Donswijk CJ, Hol SP, Hermanus C, Kuijper EJ, Lipman LJ. Aerial dissemination of *Clostridium difficile* on a pig farm and its environment. Environ Res. 2011;111:1027-32

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

Reil M, Hensgens MP, Kuijper EJ, Jakobiak T, Gruber H, Kist M, Borgmann S. Seasonality of *Clostridium difficile* infections in Southern Germany. Epidemiol Infect. 2011; 8:1-7

Hopman NE, Oorburg D, Sanders I, Kuijper EJ, Lipman LJ. High occurrence of various *Clostridium difficile* PCR ribotypes in pigs arriving at the slaughterhouse. Vet Q. 2011;31:179-81

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.

Stabler RA, Dawson LF, Valiente E, Cairns MD, Martin MJ, Donahue EH, Riley TV, Songer JG, Kuijper EJ, Dingle KE, Wren BW. Macro and micro diversity of *Clostridium difficile* isolates from diverse sources and geographical locations. PLoS One. 2012;7(3):e31559.

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. 2012 Mar 23. doi: 10.1111

Presentations and posters June 2011 – June 2012

NVMM, 17-18 april 2012

(oral). Marjolein P.M. Hensgens, Abraham Goorhuis, Olaf M. Dekkers, Daan Notermans, Birgit B.H. van Benthem, Ed J. Kuijper. Outcome nosocomial *Clostridium difficile* infections; results of a multicenter cohort study

(P) E.M. Terveer, C.W. Knetsch, D.W. Notermans, H.C. van Leeuwen, E.J. Kuijper (Leiden, Bilthoven, NL). Application of Multi Locus Sequence Typing to study the phylogenetic relationship between hypervirulent *Clostridium difficile* PRC ribotypes.

22nd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), 31 March-3 April 2012

P 1143. Hensgen MP, Dekkers OM, le Cessie S, Goorhuis A, Kuijper EJ (Leiden, Amsterdam, NL. Predicting a complicated course of *Clostridium difficile* infection using a validated scoring system at patient's bedside.

P 2286. Notermans DW, Virolainen A, Nagy E, Mastrantonio P, Ivanova K, Fitzpatrick F, Barbut F, Hall V, Kola A, Suetens S, Wilcox W, Kuijper EJ, for the ECDIS-net participants. Enhancing laboratory capacity for *Clostridium difficile* detection in Europe.

P 2228. van der Zwet WC, Wolf I, Bergervoet PWM, Sebens GW, Notermans DW, Kuijper EJ. Outbreak of *Clostridium difficile* ribotype 027 in a hospital and a nursing home, Deventer, the Netherlands

O 661. Hensgens MP, van Benthem BH, Notermans DW, Kuijper EJ on behalf of the participants of the *Clostridium difficile* national surveillance. Results of the Dutch national surveillance for Clostridium difficile infections

O 665. Verspui-van der Eijk PA, Hensgens MP, de Jong J, Frenay I, de Leeuw B, Caljouw MAA, Notermans DW, van den Kerkhof JHTC, Kuijper EJ. Emerging outbreaks of *Clostridium difficile* in regional long-term care facilities.

Invited presentations 2011-2012

12 September 2011. Keulen, University Medical Center.
Ed J. Kuijper. *Clostridium difficle* infections: old en new PCR ribotypes.

14 October 2011. **University of Edinburgh.** Oral examination PhD defense Ms Prerna Vohra; "Clostridium difficile: expression of virulence factors, resistance to disinfectants and interactions with human cells'.

23-26 October. Applying advanced molecular techniques to healthcare infections (Wellcome Trust Scientific Conference, Cambridge, UK).
Ed J. Kuijper. Changing epidemiology of *Clostridium difficle* infections in The Netherlands and Europe.

22-23 November 2011. ECDC, Stockholm, Sweden. Expert consultation: Breakthroughs in molecular epidemiology of human pathogens: how to translate into public health practice

23-25 November 2011, Warsaw. ECDC: Joint Annual Meeting of the Antimicrobial Resistance and Healthcare-Associated Infections (ARHAI) Networks
Ed J. Kuijper. European Surveillance of *Clostridium difficile* infections

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

Organization of Workshops

14 and 15 March, 2012, At Leiden University Medical Centre (ECDIS-net training module). Isolation and identification of *Clostridium difficile from* feces samples and PCR ribotyping.

31 March, 2012, London. ESGCD Pathogenesis and treatment of recurrent Clostridium difficile infections (CDI) (Educational Workshop 14)