Fifth Annual Report of the National Reference Laboratory for *Clostridium difficile* (May 2010 to May 2011) and results of the sentinel surveillance

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Acknowledgements:

We thank R. Kewalapat, S. Mooyekind, J. Hoegaerts-Goorman and M. Dittmar for their analytical support of the typing work. We also thank the administrative workers and technicians of the Department of Medical Microbiology for their contributions.

Content

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 national Reference Laboratory for *Clostridium difficile* at the Leiden University Medical Center. 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.

In the period between May 1st 2010 and May 1st 2011, 274 samples from 28 healthcare facilities and laboratories in the Netherlands were investigated at the **Reference Laboratory in Leiden**. In contrast to previous years, **type 027 was the most commonly found PCR ribotype (25.5%),** followed by type 001 (17.3%), type 014 (12.5%) and type 078 (9.6%). The increased share of type 027 was due to **two** large outbreaks. The largest outbreak due to type 027 took place in an elderly home. This outbreak is now lasting for over one year.

The results of the **sentinel surveillance in 20 hospitals** revealed that the mean incidence of CDI is 15 per 10,000 admissions, varying from 5 to 33 per 10,000 admissions. Type 001 was the most frequently found type (20%), type 014 was found in 13% and type 078 in 12%. Type 027 was found in 3%. A total of 129 patients (20%) had severe CDI. After 30 days, 7 patients (1.3%) were admitted to the ICU as a consequence of CDI; 68 patients with CDI (12.8%) died. Two deaths were attributable to CDI, 16 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 2010 and May 1st 2011 **twelve outbreaks** were seen in the sentinel surveillance and **five** in the Reference Laboratory. These outbreaks were caused by types 001, 014, 078, 087 and type 027.

No new PCR ribotypes were identified. The Reference Laboratory is now able to recognize and name 134 types. Additionally, 130 unknown types were recognized by the Reference Laboratory between 2005 and 2011. Of these types, 12 different types occurred more than 5 times and were sent to the NHS Clostridium Reference Laboratory in Cardiff, Wales for further typing. All the unknown types from 2010 were also further characterized for the presence of TcdA, TcdB and Binary toxins.

We conclude that type 027 is again becoming the predominant 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 350 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. 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 134 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 endemic situation, a new sentinel surveillance started in May 2009. Nineteen hospitals participate in this surveillance and introduced a continuous monitoring of CDI. This is the second annual report that provides an overview of the two types of surveillance conducted in the Netherlands. The previous report 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 2010 and May 1st 2011.

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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 of the Reference Laboratory

1. 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*.
2. 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.
3. To report the results of the investigation to the CIb and to medical microbiologists who submitted the samples from severe CDI diseases or outbreaks.
4. To obtain demographical data and clinical information of the patients with microbiological proven CDI.

Ad 1. 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 2. 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*.[1] All *C. difficile* strains are further investigated by PCR-ribotyping.[2] The presence of *tcdA*, *tcdB* and binary toxin genes is investigated according to standardized techniques.[3] [4] [5] Deletions in *tcd*C were determined by PCR using in-house designed primers.[6]

Ad 3. 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 4. 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.[7] [8]

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

[2] 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

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

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

[5] 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

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

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

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

Aims 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 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 previous chapter. To calculate incidence rates, we requested the participating hospitals to register 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 and *C. difficile* isolates are cultured and 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 a telephone call 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.[1] [2] 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 analyses are performed using SPSS for Windows software package, version 17.0.

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

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

Results of the Reference Laboratory

Hospitals using the Reference Laboratory

In the period between May 1st 2010 and May 1st 2011, 29 healthcare facilities and laboratories in the Netherlands sent samples to the Reference Laboratory in Leiden. Healthcare facilities participating in the Sentinel Surveillance were not included in the survey of the Reference Laboratory. In total, 274 samples were submitted of which 5% consisted of feces samples. Fecal samples were submitted by 8 of the 29 facilities. Of these 8 facilities, 2 were capable of submitting strains, the other seven facilities submitted only fecal samples.

Strains sent to the Reference Laboratory

Of the 274 samples submitted, 75.9% was positive for *Clostridium difficile* in culture. This was lower that the results of the Reference Laboratory in 2009-2010, when 84.3% of the samples submitted contained *C. difficile*. Type 027 was the most commonly found PCR ribotype (25.5%), followed by type 001 (17.3%), type 014 (12.5%) and type 078 (9.6%). The percentage of type 027 increased compared to the previous year: 2009-2010: 4.3%. Between 2005 and 2007 the incidence was higher (12% in 2006-2007 and 21% in 2005-2006). Type 001, 014 and 078 were the three most frequently found PCR ribotypes following type 027. In the previous years, these types were also frequently found.[1]

Since 1st of May 2010, five outbreaks due to three different types were observed. An outbreak was defined as >2 identical PCR ribotypes within 1 week within one department of a hospital. The largest outbreaks were caused by type 027. One outbreak started in January 2011 and is still ongoing (17 patients involved). Another outbreak among patients in 4 elderly homes started before May 2010 and is still ongoing (54 patients involved). Preliminary data report the mortality of 11 of these patients within 30 days. Ongoing research must reveal if these patients died as a consequence of CDI. Sporadic cases of type 027 were seen in one hospital.

Type 001 was responsible for two outbreaks (18 isolates and 7 isolates involved, respectively). Sporadic cases of CDI due to type 001 were seen in eight other healthcare facilities. One small outbreak was caused by type 014 (3 isolates involved). No outbreaks with type 078, were seen.

In the report of 2009-2010, one of the outbreaks with type 027 was already mentioned, but involved only 2 isolates at that time. Outbreaks caused by type 001 were seen in two hospitals between 2009 and 2010. Both hospitals did not submit samples to the Reference Laboratory this year.

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

Figure 1 (not shown): Minimal Spanning Tree of 37 PCR ribotype 027 isolates typed by multiple-locus variable-number tandem-repeat analysis (MLVA), including 19 isolates of one hospital, 17 isolates of a second healthcare facility and 1 isolate from a third hospital.
Isolates are labeled by hospital plus a number. Unique isolates or isolates with completely identical MLVA types are shown within a blue, green or orange circle. 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 grey clusters represent a large genetically related cluster, defined by an STRD $\leq$ 10. Within the grey cluster, two clonal complexes are defined by an STRD $\leq$ 2. Two isolates are not clonal or genetically related to the other isolates. **Conclusion;** two different clusters of Type 027 *C. difficile* strains from outbreaks in two Dutch healthcare facilities are very much related to each other.

Table 1: Number of isolates sent to the Reference Laboratory per location. Period: May 1st 2010 – May 1st 2011. 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	73	26,3	0	0,0	54	74,0	027	35	64,8	14	5	9,3
2	42	15,1	0	0,0	36	85,7	014	11	30,6	unknown	5	13,9
3	25	9,0	0	0,0	25	100,0	027	17	68,0	078	5	20,0
4	24	8,6	2	8,3	24	100,0	001	18	75,0	002	2	8,3
5	19	6,8	0	0,0	10	52,6	001	3	30,0	005	2	20,0
6	15	5,4	0	0,0	7	46,7	001	7	100,0	-	-	-
7	11	4,0	0	0,0	11	100,0	078	3	27,3	005	2	18,2
8	7	2,5	0	0,0	4	57,1	002	1	25,0	014	1	25,0
9	7	2,5	0	0,0	6	85,7	054	2	33,3	078	2	33,3
10	7	2,5	0	0,0	2	28,6	002	1	50,0	126	1	50,0
11	6	2,2	0	0,0	4	66,7	014	2	50,0	078	2	50,0
12	6	2,2	0	0,0	5	83,3	005	1	20,0	054	1	20,0
13	6	2,2	0	0,0	6	100,0	015	2	33,3	014	1	16,7
14	5	1,8	0	0,0	5	100,0	001	3	60,0	023	1	20,0
15	2	0,7	2	100,0	0	0,0	-	-	-	-	-	-
16	2	0,7	2	100,0	0	0,0	-	-	-	-	-	-
17	2	0,7	0	0,0	2	100,0	005	1	50,0	054	1	50,0
18	2	0,7	1	50,0	1	50,0	011	1	100,0	-	-	-
19	2	0,7	0	0,0	2	100,0	001	1	50,0	015	1	50,0
20	2	0,7	2	100,0	1	50,0	054	1	100,0	-	-	-
21	2	0,7	2	100,0	0	0,0	-	-	-	-	-	-
22	1	0,4	1	100,0	1	100,0	265	1	100,0	-	-	-
23	1	0,4	0	0,0	1	100,0	027	1	100,0	-	-	-
24	1	0,4	0	0,0	0	0,0	-	-	-	-	-	-
25	1	0,4	0	0,0	0	0,0	-	-	-	-	-	-
26	1	0,4	1	100,0	0	0,0	-	-	-	-	-	-
27	1	0,4	0	0,0	0	0,0	-	-	-	-	-	-
28	1	0,4	0	0,0	1	100,0	071	1	100,0	-	-	-
Total	**274**	**100**	**13**	**4,7**	**208**	**75,9**	**027**	**53**	**25,5**	**001**	**36**	**17,3**

* Isolates and faecal samples

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 incidence data and to identify new circulating ribotypes in the Netherlands. Therefore, we included both academic centers (n=5) and general hospitals (n=15) distributed equally in the Netherlands. In the annual report of 2009-2010, the results of 19 participating centers were described. In 2011, a new hospital joined the surveillance, therefore, twenty hospitals now participate. The geographical distribution of the participating centers is displayed in figure 1.

Between May 1st 2010 and May 1st 2011, the twenty participating centers sent in 1052 samples. These 1052 samples were all cultured and resulted in 926 (88%) *C. difficile* isolates. Type 001 was the most frequently found type, isolated in 187 of the 926 isolates (20.2%). Type 014 was found in 120 isolates (13%), type 078 in 110 isolates (12%) and type 002 in 51 isolates (6%). Twenty-four isolates were positive for type 027 (3%), 58 isolates (6%) had a PCR ribotype which pattern was not recognized in our database. The results per participating center are displayed in Table 2.

Compared to the previous year 2009-2010, no major changes were noticed. Distribution of the various PCR ribotypes was similar as with the following percentages: type 001/044 24%, type 014 14%, type 078 11%, type 002 6%. Eight isolates were positive for type 027 (2%).

Outbreaks in participating hospitals

Between May 1st 2010 and May 1st 2011, three large outbreaks and nine small outbreaks (up to 4 isolates involved) were observed in eight participating hospitals. Type 001 was responsible for all three large outbreaks in which 78 patients, 16 patients and18 patients were involved, respectively. Type 014 caused three small outbreaks. Type 078 was also responsible for three small outbreaks. A small outbreak was caused by type 027. Another small outbreak was caused by type 087.

In the previous year, type 001 was also responsible for the majority of the outbreaks. Type 014, 027 and 078 were also known to cause outbreaks in the previous years.

Incidence in participating hospitals

The incidence of CDI was measured using the data of the months in which patients were actively included. Fourteen hospitals (74%) participated actively during the whole study period (10-12 months of active participation). One hospital started the surveillance in February 2011 after which it actively participated. The remaining five hospitals actively participated in the surveillance during 3 to 8 months.

Data about number of admissions and number of admission-days were known for all participating hospitals. The mean incidence was about 15 / 10,000 hospital admissions (varying from 5 to 33 per 10,000 admissions). This incidence is 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.[1] [2]

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 693 patients included, 349 were male (50.4%) and 344 were female (49.6%). The mean age was 67 years (SD 18.6), varying from 2 to 103 years. 177 (26.6%) patients had community-onset CDI, the remaining 489 patients (73.4%) were admitted in a healthcare center when the diarrhoea started. These patients were most frequently admitted to the department of internal medicine (n=141; 31.3%) and general surgery (n=63; 14.0%). Seventeen patients were admitted to the intensive care unit (3.8%).

Most patients used antibiotics prior to the start of diarrhoea (n=406; 70.5%). In the eight weeks prior to the start of this episode of diarrhoea, 63 patients (14.8%) had an episode of CDI.

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

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

A total of 129 patients (20.2%) 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, 456 patients (85.9%) had an uncomplicated course of their CDI infection. After 30 days, 7 patients (1.3%) were admitted to the ICU as a consequence of CDI; 68 patients with CDI (12.8%) died. Two deaths were attributable to CDI, 16 deaths were contributable to CDI. Half of these patients had CDI due to type 001. Other types involved in attributable or contributable death were type 014, 005, 228, 045.

Figure 2: Participating hospitals of the sentinel surveillance

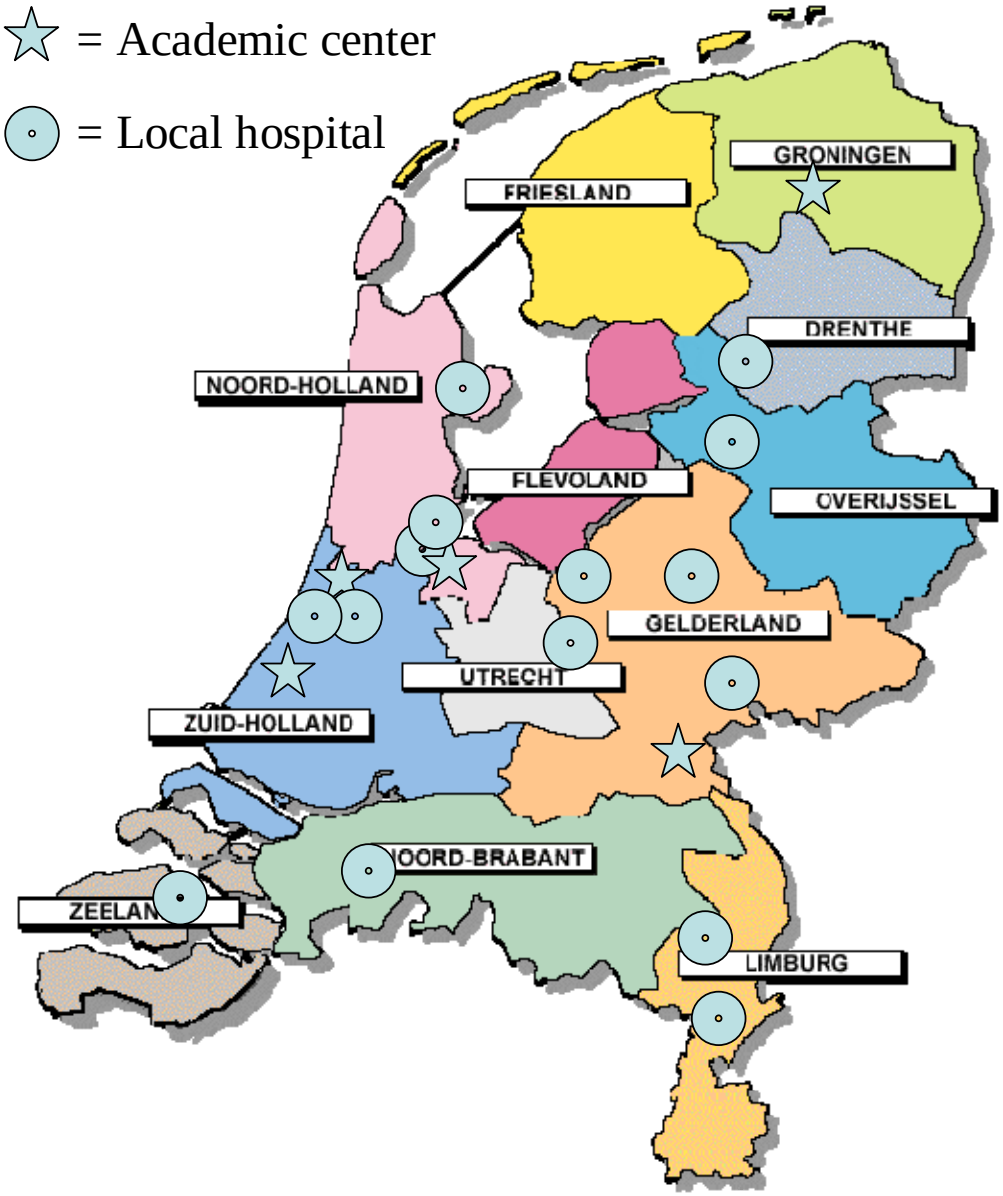


Table 2: Isolates submitted for PCR ribotyping by hospitals participating in the sentinel surveillance. Period: May 1st 2010 – May 1st 2011.

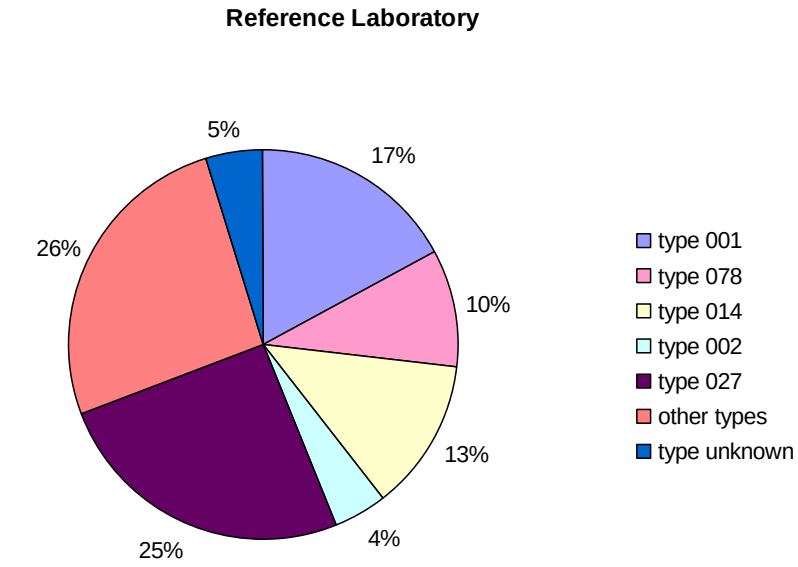
	Samples*		Faeces		*C. difficile*		Most common type			2nd most common type		
Participating hospital	**(N)**	**(%)**	**(N)**	**(%)**	**(N)**	**(%)**		**(N)**	**(%)**		**(N)**	**(%)**
A	141	13,4	8	5,7	125	88,7	001	78	62,4	014	11	8,8
B	50	4,8	0	0,0	45	90,0	078	13	28,9	002	3	6,7
C	0	0,0	0	0,0	0	0,0	-	-	-	-	-	-
D	38	3,6	3	7,9	37	97,4	015	6	16,2	014	5	13,5
E	77	7,3	0	0,0	70	90,9	001	24	34,3	014	14	20,0
F	42	4,0	0	0,0	41	97,6	001	9	22,0	087	5	12,2
G	51	4,8	48	94,1	38	74,5	001	11	28,9	014	4	10,5
H	19	1,8	0	0,0	15	78,9	014	4	26,7	078	4	26,7
I	41	3,9	0	0,0	41	100,0	005	10	24,4	078	7	17,1
J	68	6,5	0	0,0	67	98,5	001	30	44,8	027	9	13,4
K	4	0,4	1	25,0	2	50,0	002	1	50,0	122	1	50,0
L	64	6,1	0	0,0	50	78,1	002	8	16,0	014	8	16,0
M	4	0,4	0	0,0	3	75,0	002	1	33,3	009	1	33,3
N	94	8,9	0	0,0	87	92,6	001	13	14,9	014	12	13,8
O	15	1,4	0	0,0	13	86,7	014	4	30,8	002	1	7,7
P	43	4,1	30	69,8	32	74,4	027	8	25,0	078	4	12,5
Q	30	2,9	30	100,0	23	76,7	014	6	26,1	078	4	17,4
R	65	6,2	58	89,2	42	64,6	078	14	33,3	014	7	16,7
S	6	0,6	3	50,0	4	66,7	002	1	25,0	005	1	25,0
T	200	19,0	1	0,5	188	94,0	014	28	14,9	078	21	11,2
Total	**1052**	**100**	**182**	**17,3**	**926**	**88,0**	**001**	**187**	**20,2**	**014**	**120**	**13,0**

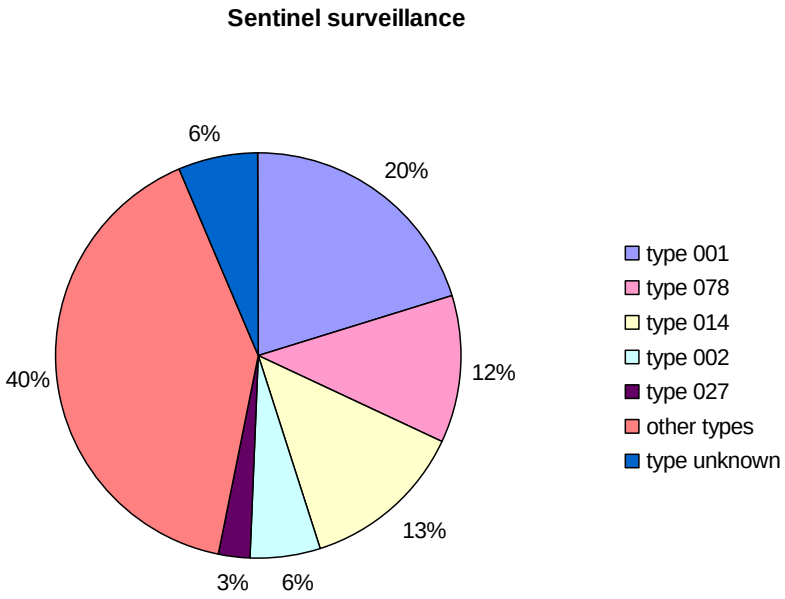
* Isolates and faecal samples

Table 3: Number of patients included in the sentinel surveillance per location and incidence data. Period: May 1st 2010 – May 1st 2011. The incidence data were calculated using only the months of active participation.

Participating hospital	Months of active participation	Patients (N)	Patients (%)	Monthly admissions	Monthly admission-days	Incidence / 10,000 admissions	Incidence / 10,000 admission-days
A	8	70	10%	2621	12905	33,38	6,78
B	5	15	2%	2360	16642	12,71	1,80
C	8	15	2%	1077	6499	17,41	2,89
D	3	5	1%	2229	10627	7,48	1,57
E	2	10	1%	3082	14524	16,22	3,44
F	11	45	6%	2421	11330	16,90	3,61
G	12	51	7%	2839	15107	14,97	2,81
H	12	17	2%	1959	10902	7,23	1,30
I	12	41	6%	2886	23253	11,84	1,47
J	10	44	6%	1532	7131	28,72	6,17
K	12	10	1%	1537	7700	5,42	1,08
L	12	38	5%	1710	11537	18,52	2,74
M	12	7	1%	1024	4684	5,70	1,25
N	12	81	12%	3059	15266	22,07	4,42
O	11	8	1%	1213	6511	6,00	1,12
P	12	30	4%	3387	24905	7,38	1,00
Q	5	11	2%	2241	12144	9,82	1,81
R	12	63	9%	2580	15992	20,35	3,28
S	12	7	1%	859	4301	6,79	1,36
T	12	125	18%	3946	19045	26,40	5,47
Total		**693**	**100**	**44562**	**251005**	**14,76**	**2,77**

Figure 3: Percentage of the 6 most frequently encountered PCR ribotypes. Reference Laboratory data and data from the sentinel surveillance are displayed apart. Period: May 1st 2009 – May 1st 2010. The category 'other types' consists of 52 different PCR-ribotypes in the Reference Laboratory data and 42 different types in the Sentinel surveillance data.





Conclusions and recommendations

- The Reference laboratory is now capable to recognize 134 different PCR ribotypes. We are in the process of obtaining more ribotypes by sending our unknown types that occurred more than 5 times in our database, to the NHS Clostridium Reference Laboratory in Cardiff, Wales
- In the period between May 1st 2010 and May 1st 2011, 286 samples from 29 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. In contrast to previous years, type 027 was the most commonly found PCR ribotype (25.5%), followed by type 001 (17.3%), type 014 (12.5%) and type 078 (9.6%). The increased share of type 027 was due to two large outbreaks.
- Of the 274 samples submitted to the Reference Laboratory, 75.9% was positive for *Clostridium difficile* in culture. This was lower that the results in 2009-2010, when 84.3% of the samples submitted contained *C. difficile*. Since this observation, all negative cultures (samples that are send to the Reference Laboratory and samples from the sentinel surveillance) are also examined by a second technician. This resulted in that 8.4% of the negative cultures were in fact positive for *Clostridium difficile*.
- The results of the sentinel surveillance in 20 hospitals revealed that the mean incidence of CDI is 15 per 10,000 admissions, varying from 5 to 33 per 10,000 admissions.
- The largest outbreak due to type 027 (encompassing 54 patients) took place in an elderly home. Because this outbreak is now lasting for over one year and outbreaks in elderly homes seem hard to tackle, we would like to investigate this in more detail. Therefore, we recommend to obtain more data from the sentinel surveillance programme and to extend the number of participating facilities with representative nursing homes.
- The results of the sentinel surveillance in 20 hospitals, revealed that the mean incidence of CDI is 15 per 10,000 admissions, varying from 5 to 33 per 10,000 admissions. Type 001 was the most frequently found type (20%), type 014 was found in 13% and type 078 in 12%. Type 027 was found in 3%. A total of 129 patients (20%) had severe CDI. After 30 days, 7 patients (1.3%) were admitted to the ICU as a consequence of CDI; 68 patients with CDI (12.8%) died. Two deaths were attributable to CDI, 16 deaths were contributable to CDI.
- During the period of sentinel surveillance, twelve outbreaks were observed due to types 001, 014, 078, 087 and type 027.
- The incidence of CDI in the Netherlands is unchanged compared to 2009-2010 with *C. difficile* type 078 as the third most frequent found type. 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.
- *C. difficile* type 078 has similar virulence markers as type 027 (producing toxins A and B, binary toxin positive, a mutation in tcdC and leading to a stop codon and a deletion in TCdC) and has been found widespread in The Netherlands. Its association with neonatal diarrhoea in piglets is currently investigated in a ZonMW project in collaboration with the University of Utrecht.

[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