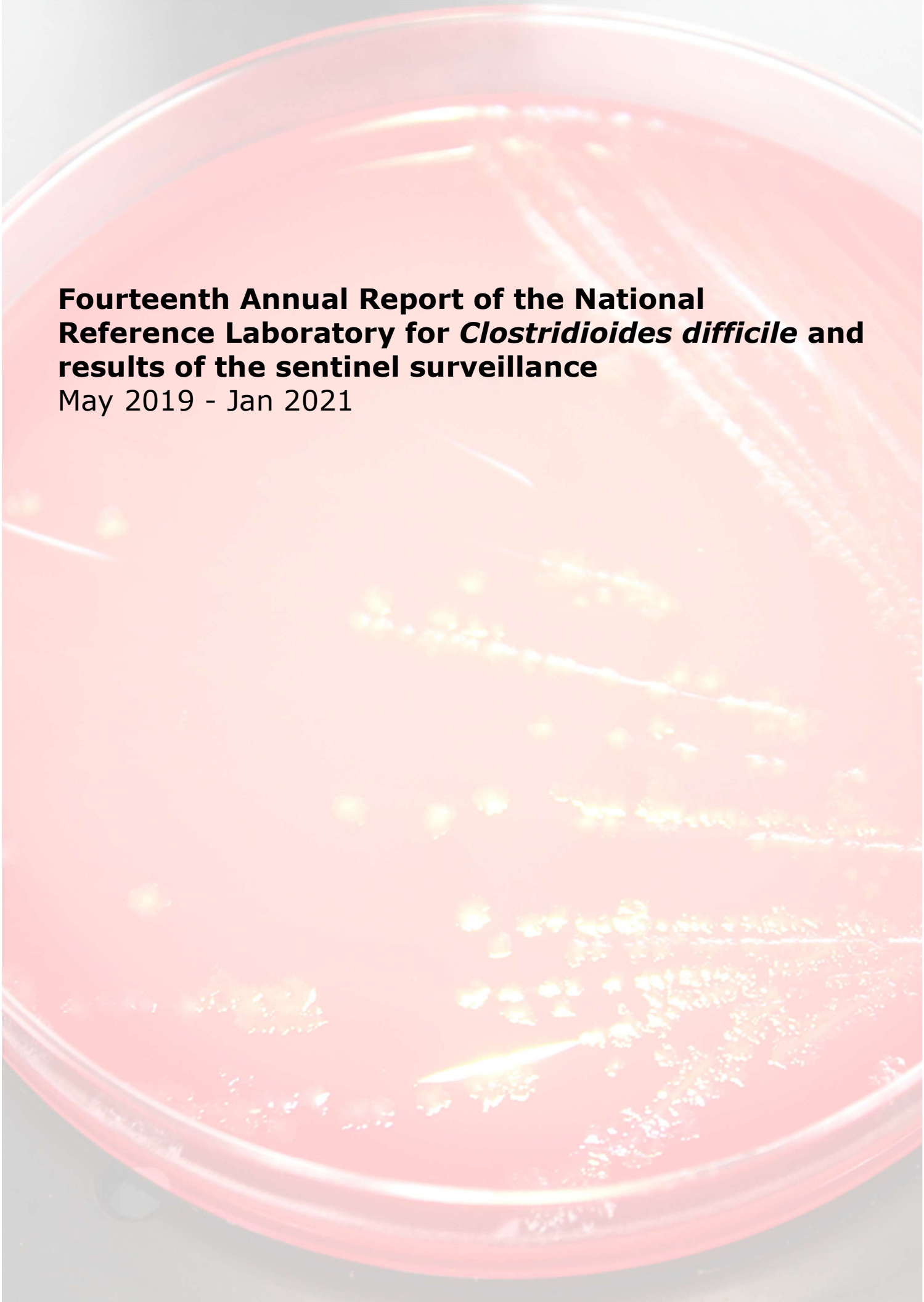

Fourteenth Annual Report of the National Reference Laboratory for *Clostridioides difficile* and results of the sentinel surveillance

May 2019 - Jan 2021

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Content

Introduction

Clostridioides difficile (C. difficile) is an anaerobic, spore-forming bacterium which can colonise the intestine of humans and animals. Pathogenic *C. difficile* strains can produce protein toxins (toxin A and/or B, and/or binary toxin) that disrupt the intestinal wall and thereby cause mild diarrhoea, severe colitis or a life-threatening toxic megacolon depending on host susceptibility and the virulence of the infecting strain.[1]

Diagnosis

The diagnosis of *C. difficile* infection (CDI) is most frequently based on clinical signs and symptoms in combination with laboratory tests. In 2016, a revision of the ESCMID guidelines on CDI diagnosis was published.[2] According to these guidelines the use of a two-step algorithm to diagnose CDI is recommended. These guidelines also stress the fact that a distinction between CDI patients and *C. difficile* carriers is not possible if only tests that detect the toxin-producing potential (i.e. toxin B PCR or toxigenic culture) are used instead of the detection of free toxins present in stools (i.e. by toxin A/B enzyme immunoassay). The ECDC surveillance protocol for CDI also recommends to use a two–step algorithm. Alternatives to laboratory diagnosis are endoscopy or histopathology to diagnose pseudomembranous colitis, though this diagnosis is not specific for CDI and is also associated with other enteropathogens or medication effects. Cultured isolates can be subtyped by PCR ribotyping. PCR ribotyping uses the PCR ribotype-dependent differences in profiles generated by PCR amplification of the intergenic spacer regions between the 23S and 16S rRNA genes.[3] The Reference Laboratory is currently able to recognise 306 different PCR ribotypes and is exploring application of whole genome sequencing (WGS) for typing of *C. difficile*.

Transmission and infection control

Transmission of *C. difficile* within the hospital setting is common. However, the changing view is that *C. difficile* is not only transmitted by symptomatic CDI patients. Asymptomatic carriers can also introduce the bacterium into the hospital and spread it to other patients, although at a lower rate than symptomatic CDI patients.[4,5] Data from a large cohort study in three hospitals in the Netherlands suggests that this is different in a low-endemic setting (Crobach et al. submitted 2021). Yet, standard infection control precautions focus on CDI patients only. The national WIP guideline (July 2011) recommends application of contact precautions in combination with hospital cleaning and disinfection[6], though many Dutch hospitals do not enforce the use of high concentrations of chloride due to occupational health issues. Antibiotic stewardship is another important factor in reducing CDI incidence.[7] At the moment, detecting and isolating *C. difficile* carriers is not generally recommended. Also, most hospitals stop contact precautions 48 hours after the last diarrhoeal symptoms, although it is known that CDI patients may shed spores for a prolonged amount of time.[8]

In 2018, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) study group for *C. difficile* (ESGCD) published an updated guideline on CDI prevention[9] using a systematic review of the literature. Screening for *C. difficile* to identify colonised/carrier patients or healthcare workers was not recommended by the study group. For hand-hygiene, it is recommended to switch from alcohol-based hand rub (AHR) to washing with soap and water in an outbreak setting, but not in an endemic situation. Contact precautions and the use of personal protective equipment (PPE), such as gloves and gowns/disposable aprons, are also advised to decrease transmission of *C. difficile*. Daily and terminal environmental sporicidal disinfection should be applied in rooms of patients with CDI to eradicate spores. To achieve a reduction of the CDI rates in hospitals, the study group recommends the restriction of antibiotic agents/classes and a reduction of the duration of antibiotic therapy. The study group concludes that education for health care workers, environmental service personnel, CDI patients and visitors on prevention of CDI is very useful.

Treatment of *C. difficile* infection

The first step in the management of CDI is to discontinue the inciting antibiotic, if possible. Antibiotic treatment of CDI (with either metronidazole, vancomycin or fidaxomicin) is tailored by severity of disease and also differs for an initial episode, single recurrence or multiple recurrences.

Metronidazole is commonly used, but metronidazole resistance is increasingly being reported[10] and may be associated with clinical failure.[11] Importantly, metronidazole resistance may be underestimated due to the medium-dependent MIC results.[10,12]

In September 2021, the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA) published Clinical Practice Guidelines for CDI, which recommend

fidaxomicin over metronidazole and vancomycin as treatment for an initial episode or recurrent CDI. Furthermore, bezlotoxumab is recommended for recurrent CDI or for a primary CDI episode with risk factors for recurrence in addition to standard-of-care antibiotics.[13] In 2021, new ESCMID guidelines on the treatment of CDI have been endorsed, in which metronidazole is excluded and in which fidaxomicin and bezlotoxumab (humanised monoclonal antibodies against *C. difficile* toxin B) have a more prominent place to prevent recurrences.[14]
Despite antibiotic therapy, CDI recurrence is common. Faecal microbiota transplantation is proven to be very effective as treatment for recurrent CDI, likely by restoring the healthy gut microbiota.[15] Due to the high costs and time-consuming nature of donor screening, faecal microbiota transplantation was earlier frequently not offered despite an indication for it. To overcome these problems, the National Donor Faeces Bank (NDFB) was set up at Leiden University Medical Centre in 2016 (http://www.ndfb.nl/). The aim of the NDFB is to make transplantation of carefully screened donor faeces easily available for treatment of patients with multiple relapsing CDI.[16] Donors are healthy volunteers who are screened according to a standardised protocol including questionnaires and microbiological investigations of serum and faeces. Stool preparations of these healthy donors are stored at the biobank in the LUMC. These ready-to-use frozen donor faeces suspensions can be ordered by treating physicians of patients with recurrent or severe CDI (info@ndfb.nl). Patients can receive the faecal microbiota transplantation at their local hospital. A total of n=143 faecal microbiota transplantations for recurrent CDI with a faeces suspension from the NDFB were performed in the period May 2016-August 2019 with a cure rate of 89% after two months.
Recently, bezlotoxumab has been tested in a clinical setting to prevent recurrent CDI.[17] More evidence is becoming available that suggests that bezlotoxumab may be an alternative option for the prevention of recurrent CDI, mainly in patient with risk factors for recurrence.[18,19] In 2021/2022, a study will start to compare the efficacy of FMT with bezlotoxumab.

Epidemiology
Before 2005, CDI outbreaks were rarely reported in the Netherlands. In 2005, the *C. difficile* ribotype 027 strain (or NAP1/REA BI strain) was for the first time detected[20] and rapidly spread within Netherlands while causing major outbreaks.[21,22] Retrospectively, the rapid spread of the ribotype 027 strain across Northern-America and Europe has been attributed to its high level of fluoroquinolone resistance.[23] A study by *Collins et al* suggests that the rapid spread might also be attributed to a different trehalose metabolism in ribotype 027 strains, which causes the ability to metabolise low concentrations of trehalose. The implementation of trehalose as a food additive into the human diet, shortly before the emergence of ribotype 027, might have stimulated the spread of ribotype 027.[24] CDI cases due to ribotype 027 were associated with unfavourable patient outcomes such as severe disease, mortality and recurrent CDI in comparison to other ribotypes[21,25], which may reflect type-specific host susceptibility and/or an increased virulence of the strain.[26] Since mid-2006, the occurrence of ribotype 027 in the Netherlands has decreased significantly.[27] The CDI incidence rate has stabilised at 3 CDI cases per 10,000 patient-days.[28]
Interestingly, more ribotype 027-like strains are beginning to emerge, like ribotype 036, 198 and 181. They are mainly observed in Eastern-Europe. In 2019, four isolates with ribotype 198 were found in one Dutch hospital, while this ribotype has only been observed once before in the Netherlands by the Dutch Reference laboratory.

Surveillance and ad hoc typing
The Centre for Infectious Disease Control (CIb) of the National Institute for Public Health and the Environment (RIVM) started a National Reference Laboratory for *C. difficile* at the Leiden University Medical Centre soon after recognition of *C. difficile* ribotype 027 outbreaks in 2005. Since then, this laboratory has offered ad hoc typing service for all microbiology laboratories in the Netherlands for typing of *C. difficile* isolates of patients with severe disease, or isolates from a suspected outbreak. Additionally, the National Reference Laboratory initiated a sentinel surveillance programme in May 2009 to monitor the incidence of CDI in an endemic situation. Furthermore, the programme aims to monitor (new) emerging strains of *C. difficile*. In 2021, the surveillance activities of the Reference laboratory will decrease, but the preparedness of a rapid response in outbreaks will continue in the form of an "Expertise Centre" located both in the LUMC and RIVM.
In the period of May 2019 until Jan 2021 twenty-two acute care hospitals participated in the sentinel surveillance programme voluntarily. Each year, results are reported on the website of the National Institute for Public Health and the Environment (RIVM).[28] This current report is the fourteenth annual report that provides an overview of the two types of surveillance conducted in the Netherlands, describing the situation in the Netherlands between May 1st 2019 and Jan 1st 2021. The surveillance period is not

from May to May this year, which has been the case in the precious years, due to the transition of the surveillance period towards that of the ECDC surveillance.

The Netherlands is also participating in the European-wide CDI surveillance which is led by ECDC. For this, the data of all participating hospitals of the Dutch sentinel surveillance is sent to ECDC once every year. The protocol for this European surveillance program is available at: https://www.ecdc.europa.eu/en/publications-data/european-surveillance-clostridium-difficile-infections-surveillance-protocol-2.

Aims and procedures of the sentinel surveillance

The aims of the national sentinel surveillance of *C. difficile* infections are:

1. To obtain continuous incidence rates of patients with CDI in participating hospitals in the Netherlands.
2. To identify and characterise new circulating PCR ribotypes.
3. To correlate newly found circulating PCR ribotypes with changes of epidemiology and clinical syndromes of CDI.

Patient inclusion
Hospitals participating in the sentinel surveillance are requested to include in the surveillance all hospitalised patients with clinical signs or symptoms of CDI in combination with a positive test for *C. difficile* toxins or toxigenic *C. difficile*. Before May 2019 patients aged <2 year were not included and after May 2019 these were included in case of a strong clinical suspicion of CDI. Patients are tested on their physicians' request or without a specific request, for instance if they are admitted to the hospital for three days or more and their unformed stool is submitted to the laboratory (the "three day rule"). The assay or algorithm that is used to diagnose CDI, is chosen by the local laboratory. Laboratories that culture *C. difficile* (n=10) send strains to the laboratory of the Leiden University Medical Center. Other laboratories (n=9) send faecal samples. Some laboratories (n=3) send faecal samples or strains.

Collection of patient data
The OSIRIS system is used to complete a web-based questionnaire for each included patient. This questionnaire contains questions involving e.g. patient's gender, age, location of onset of the infection, symptoms of the infection and antibiotic use. Furthermore, the outcome after 30 days is requested. The definitions applied in this questionnaire are based on those proposed by the ECDC and the CDC.[29,30] In the OSIRIS system, the results of the PCR ribotyping (completed by the reference laboratory) 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 by all participating individual laboratories for their own data.

Microbiological reports
All faecal samples are cultured and *C. difficile* isolates are characterised (see next chapter) at the laboratory of the Leiden University Medical Centre. In case PCR ribotype 027 (or a 027-like ribotype) is found, the local microbiologist is directly informed by telephone and asked if there is a need for additional information or advice. If there is suspicion of an outbreak, laboratories will also be contacted as soon as possible by telephone and/or e-mail. Once a week, microbiological results are sent by e-mail to the submitting microbiologist, infection control practitioners, and, when an outbreak is suspected or ribotype 027 isolated, also to CIb. The results are also reported in OSIRIS. All submitting laboratories also receive the official report by regular post. Once a year, an overview of the results of the sentinel surveillance is provided to the participating hospitals by a national report and a special meeting organised by the LUMC and RIVM.

Incidence rates and outbreaks
The last data-extraction for this annual report was performed on June 22th 2021. To calculate incidence rates, we requested the participating hospitals to register their monthly number of admissions and number of patient-days. Usually, if no data were available, the data from the previous year were used as denominator. However, due the COVID-19 pandemic in 2020, we assume that the numbers of the previous year are not representative and hospitals with missing data were therefore excluded from incidence rate calculations. Incidence rates are estimated by the number of CDI patients per 10,000

patient-days. The 95% confidence intervals for incidence rates were calculated by assuming a Poisson distribution.[31]
A suspected outbreak was defined if >2 isolates of the same type were found less than 7 days apart in one hospital, either with onset of symptoms on the same department, or accompanied with an increased CDI monthly incidence within the hospital. If Multiple-Locus Variable number tandem repeat Analysis (MLVA) is performed, there should be an STRD≤10 to determine the isolates as genetically related, also dependent on the PCR ribotype. Using whole genome multilocus sequence typing (wgMLST), the threshold is lower and set to three alleles differences (Baktash et al, submitted 2021). Statistical analysis were performed using Excel and STATA/SE for Windows software package, version 15.1. Maps were created through FreeVectorMaps.com.

Aims and procedures of the ad hoc typing

The aims of the ad hoc typing are:

1. To provide medical microbiological laboratories not participating in the sentinel surveillance the opportunity to have *C. difficile* strains isolated and typed in case of suspected outbreaks in hospitals or other health care facilities.
2. To isolate *C. difficile* for further typing from faecal samples of patients with CDI sent to the reference laboratory by laboratories that do not culture *C. difficile*.
3. To characterise isolated *C. difficile* strains by PCR ribotyping, presence of genes *tcd*A and *tcd*B, presence of binary toxin genes and the presence of deletions in *tcd*C.
4. To investigate the relatedness of the isolates by MLVA or wgMLST.[32,33]
5. To report the results of the investigation to CIb and to medical microbiologists who submitted the samples from severe CDI diseases or outbreaks.
6. To obtain demographical data and clinical information of the patients with CDI.

C. difficile isolation

Isolation of *C. difficile* from faecal samples at the Reference laboratory is performed on *C. difficile* selective agar supplemented with cefoxitin, amphotericin B and cycloserine (CLO-medium; BioMérieux), with and without ethanol shock pre-treatment. After incubation in an anaerobic environment at 37 °C for 48h, colonies of Gram-positive rods with subterminal spores are tested for the presence of the glutamate dehydrogenase gene by an in-house PCR.

C. difficile confirmation

All isolates are genetically identified as *C. difficile* by an in-house PCR for the presence of the *gluD* gene, encoding the glutamate dehydrogenase (GDH) specific for *C. difficile*.[30] All *C. difficile* strains are further investigated by PCR-ribotyping.[3] The presence of *tcdA*, *tcdB* and binary toxin genes is investigated by multiplex PCR on request.[34] Deletions in *tcdC* can be determined by PCR using in-house designed primers.

C. difficile Reference Library

The Reference Laboratory added 33 new ribotypes to the Reference Library in the period may 2019 – Jan 2021, and is now able to recognise 306 different PCR ribotypes. If an unknown ribotype is isolated more than five times, the electronic capillary PCR ribotyping profiles and data of WGS are send to the Department of Microbiology, Leeds Teaching Hospitals NHS Trust, Leeds (dr. Warren Fawley, prof. Mark Wilcox), to assign a (new) ribotype.

Microbiological reports

Results of microbiological analysis are sent by e-mail to the submitting microbiologist and to CIb. When PCR ribotype 027 (or a 027-like ribotype) is found, the laboratories are also informed by telephone and are offered to contact the LUMC or CIb for additional information and advice. Submitting laboratories also receive an official report by regular post.

Collection of patient data

A standardised questionnaire is used to obtain information on patient's age and gender, the ward where CDI was acquired, clinical data, risk factors, antibiotic treatment in the month preceding a positive test and treatment outcomes. The definitions applied in this questionnaire are based on those proposed by the ECDC and the CDC.[29,30] Co-morbidity is defined according to the ICD-10 classification.

The questionnaires are sent by e-mail to the submitting laboratories when faecal samples or isolates are received.

The COVID-19 pandemic

The surveillance period covered in the current annual report is different than for previous reports, since the COVID-19 pandemic occurred in 2020. This led to a decrease in the number of hospital patients and a different patient population in hospitals with the majority being patients with COVID-19 patients, due to the high incidence of COVID-19 and due to the restricted referral to hospitals. We assessed whether this led to a different CDI epidemiology. The period of the COVID-19 pandemic was subdivided into the first wave (13th of March 2020 until the 12th of May 2020), the second wave (17th of September 2020 until the 1st of January 2021) and the interwave (period in between). The periods were based on the signal value of the National Institute of Public Health and the Environment, i.e. waves were assigned for periods in which the mean number of hospital admissions per day of the previous seven days was more than 40. Characteristics of CDI patients with a sample date within these periods and the monthly CDI incidence rates were compared between 2020 and 2019 or 2015-2019. Both 2019 and 2015-2019 were included in the comparison to facilitate the distinction between a true COVID-19 pandemic effect and trends in time. Incidence rates were compared using Poisson regression analysis and incidence rate ratios (IRR) with 95% CI were calculated. Categorical variables were analysed using Pearson's chi-squared test or, in case of an expected count of cells of <5, the Fisher's exact test and were accompanied by odds-ratios (OR) with 95% CI.

Results of the sentinel surveillance

Participating hospitals

This section describes the results of the 22 participating hospitals of the sentinel surveillance programme in the period May 2019 - Jan 2021. Both university hospitals (n=5) and primary or secondary care hospitals (n=17) were included, distributed all over the Netherlands. The geographical location of the participating hospitals is displayed in Figure 1.

Figure 1. Participating hospitals of the sentinel surveillance between May 2019 and Jan 2021. University hospitals are depicted in orange, primary/secondary care hospitals are depicted in blue

Most laboratories did not often receive test requests for CDI from general practitioners: three often (> 1 per day), nine sometimes (1 or more per week or per month), two sporadically (less than 1 per month), four never and of four laboratories it was unknown whether they received test requests from general practitioners.

Diagnostic testing

The diagnostic tests used by the participating hospitals to diagnose CDI are depicted in Table 3 and Figure 2. By Jan 2021, 13/22 hospitals (59.1%) used an ESCMID recommended algorithm (algorithms A and B), which was 52.1% in last year. Another eight hospitals (36.4%) used a stand-alone nucleic acid amplification test (NAAT) which is either a PCR or a loop-mediated isothermal amplification (LAMP) assay to detect toxin A and/or B genes. Four of the eight hospitals relying on NAAT performed culture on NAAT-positive samples for confirmation and to have the isolates available for typing. One hospital used an GDH Enzyme Immunoassay and, when positive, NAAT. Eight out of 22 hospitals (36.4%) tested unformed stool samples from patients 2 years or older admitted for at least 3 days (the so-called "three day rule") or with a specific request for CDI testing. Nine of the 22 hospitals (40.9%) tested submitted stool samples for CDI in case of a request for CDI testing only, and two more only if the stool sample was unformed. In 14 hospitals, restrictions applied for CDI testing of stool samples from young children (<2 years) (Figure 3). The mean percentage of *C. difficile* positive patients among all patients tested was 7.9% (range 1.6-51.2%; Table 3).

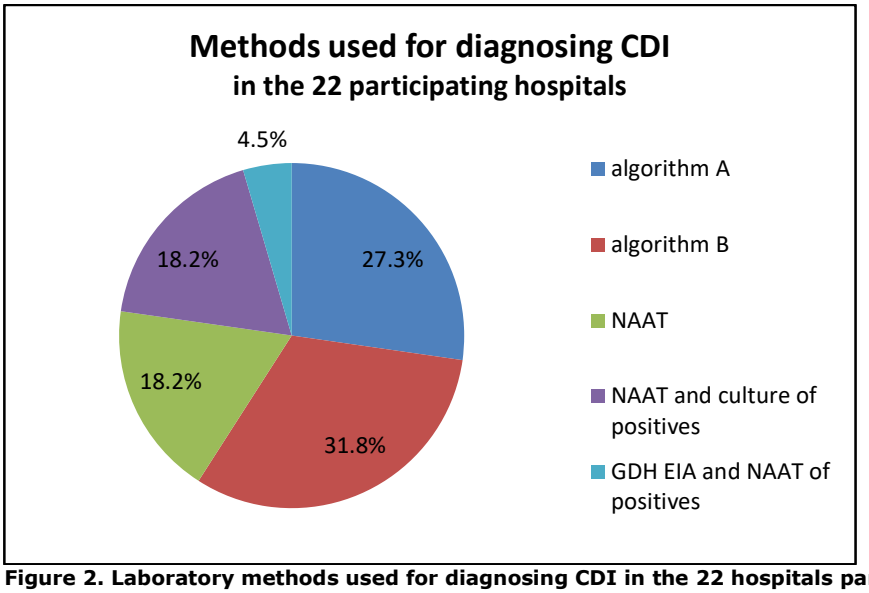


Figure 2. Laboratory methods used for diagnosing CDI in the 22 hospitals participating in the sentinel surveillance program. Algorithm A (NAAT or GDH EIA - Tox A/B EIA) and B (GDH & Tox A/B EIA, and in some hospitals confirmation with NAAT/toxigenic culture) are ESCMID-recommended methods, all the others are non-recommended methods. Two hospitals that use NAAT (and culture) used a different diagnostic method in the weekend (GDH and toxin EIA, and in one hospital confirmation with NAAT when not conclusive).

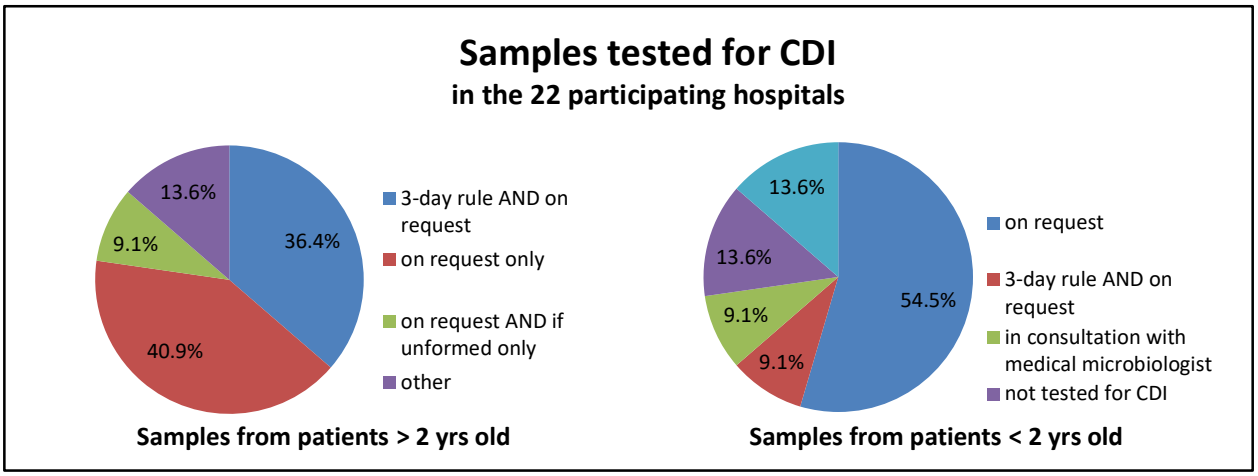


Figure 3. Samples tested for CDI in the 22 hospitals participating in the sentinel surveillance. Selection criteria for samples from patients >2 years are shown on the left, selection criteria for samples from patients <2 years are shown on the right.

Incidence in participating hospitals
The numbers of CDI per 10,000 patient-days per hospital are shown in Table 3, and are compared to the incidence rate of the preceding year. The mean incidence was 3.20 CDI cases per 10,000 patient-days (varying from 1.91 to 5.23 CDI cases per 10,000 patient-days), comparable to the incidence of 3.17 that was reported in May 2018 – May 2019.[28]

Submitted strains for PCR ribotyping
Of 1382 CDI patients included in sentinel surveillance between May 1st 2019 and Jan 1st 2021, 1058 (76.6%) *C. difficile* isolates could be PCR ribotyped and linked to the clinical data. The most important reasons for missing data were the inability to culture *C. difficile* at the local laboratory, refrainment from sending the isolates or faeces to the National Reference Laboratory (n=209), no registration of the patient in OSIRIS (n=unknown) or the inability to type *C. difficile* at the National Reference laboratory (culture negative or other *Clostridium* species; n=115).

Circulating PCR ribotypes
Similar as the previous year, ribotype 014/020 was the most frequently isolated ribotype.
This year ribotype 002 was the second most frequently isolated ribotype, in contrast to last year when this was ribotype 078/126.
Ribotype 014/020 (014 and 020 are indistinguishable by conventional PCR ribotyping) was isolated in 192 of the 1058 samples (18.1%; 95% CI 15.8-20.5). Ribotype 002 was found in 105 isolates (9.9%; 95% CI 8.1-11.7). Ribotype 078/126 was found in 92 isolates (8.7%; 95% CI 7.0-10.4), ribotype 001 in 74 isolates (7.0%; 95% CI 5.5-8.5), and ribotype 005 in 70 isolates (6.6%; 95% CI 5.1-8.1). Two isolates were identified as ribotype 027 (0.2%; 95% CI 0.0-0.5). Of 67 isolates (6.3%; 95% CI 4.9-7.8) the PCR ribotype pattern was not recognised in our database. Of these isolates, 11 groups of unknown ribotypes were exactly the same, of which nine were pairs of two isolates and one group included 10 isolates and one group included four isolates. The results stratified per participating centre are displayed in Table 4. A pie-chart of the five most common ribotypes and the hypervirulent ribotypes of patients included in the sentinel surveillance is illustrated in Figure 7.

Changes in circulating PCR ribotypes
The proportion of the five most common ribotypes in time is shown in Figure 4.
Ribotype 014/020 had a proportion of 11.9% (95% CI 9.1-14.7) at the start of the sentinel surveillance in May 2009-May 2010, which increased to 18.1% (95% CI 15.8-20.5) in May 2019-Jan 2021. The proportion of May 2018-May 2019 was 19.5% (95% CI 16.6-22.4). The proportion of ribotype 002 was also increased compared to the start of the surveillance (May 2009-May 2010 5.6% (95% CI 3.6-7.5), May 2019-Jan 2021 9.9% (95% CI 8.1-11.7)). The proportion of May 2018-May 2019 was 9.6% (95% CI 7.5-11.8). The proportion of ribotype 078/126 was 11.7% (95% CI 9.3-14.1) in May 2018-May 2019 and 8.7% (95% CI 7.0-10.4) in May 2019-Jan 2021. The proportion of RT001 was 26.5% (95% CI 22.7-30.3) at the start of the surveillance in May 2009-May 2010, which is higher than 7.0% (95% CI 5.5-8.5) in May 2019-Jan 2021. In 2016-2017, there was an outbreak of ribotype 001 with an increased proportion of ribotype 001.
The proportion of ribotype 027 was not different from last year (May 2019-Jan 2021 0.0-0.5; May 2018-May 2019 95% CI 0.0-1.1). The proportion remained lower compared to the surveillance periods in May 2009-May 2014 (2009-2010 95% CI 2.5-6.0, 2010-2011 95% CI 1.1-3.6, 2011-2012 95% CI 1.1-3.4, 2012-2013 95% CI 2.0-4.8, 2013-2014 95% CI 1.9-4.6, see Figure 5). Ribotype 027 was found in two individual cases in two hospitals (2/24; 8.3%).

(Suspected) outbreaks in participating hospitals
In the period between May 1st 2019 and Jan 1st 2021, no clusters of *C. difficile* in hospitals participating in the sentinel surveillance were reported to the National Reference Laboratory.
In December 2019/January 2020, there was an increase in RT002 in two hospitals with several epidemiological links (n=7 and n=6). However, for one hospital none of the isolates were related based on MLVA and for the other hospital two isolates were genetically related of the four isolates that were included. These are no clusters according to the definition. Notably, there was an increase noticed in the incidence of RT002 in that period in other European countries. One hospital had six cases with *C. difficile* ribotype 198 in different months in 2019 (no cluster according to the definition), of which four were identical or clonal complexes with MLVA. This ribotype has only been observed once before in the Netherlands.

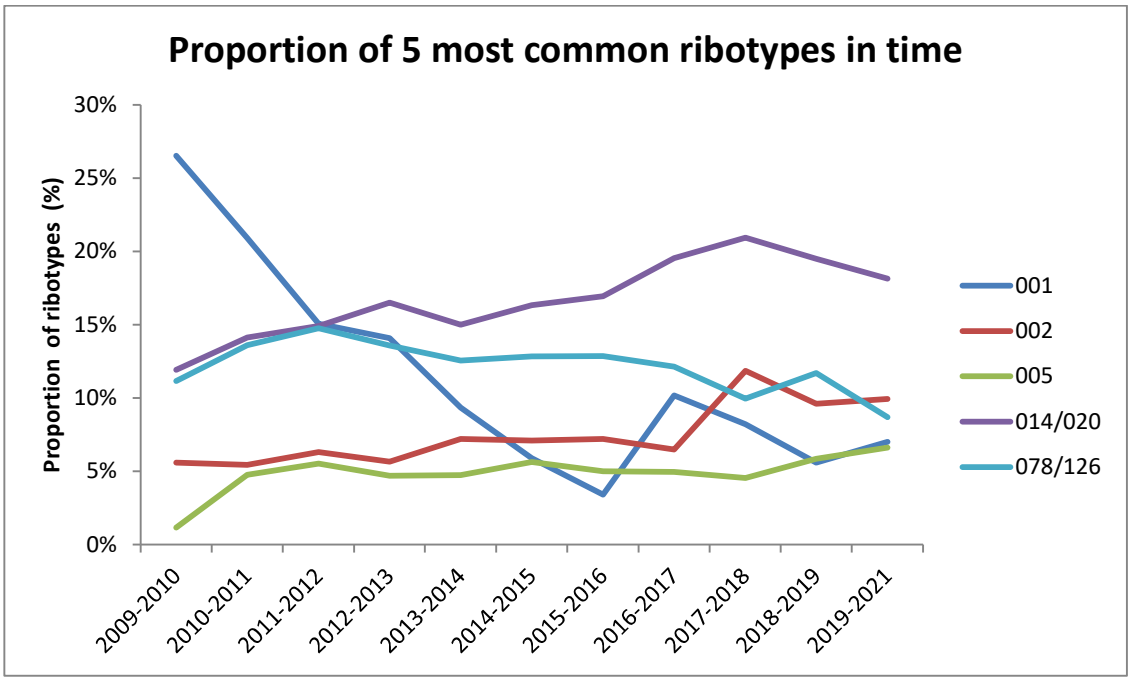


Figure 4. Proportions of the 5 most common ribotypes in time in sentinel surveillance samples.

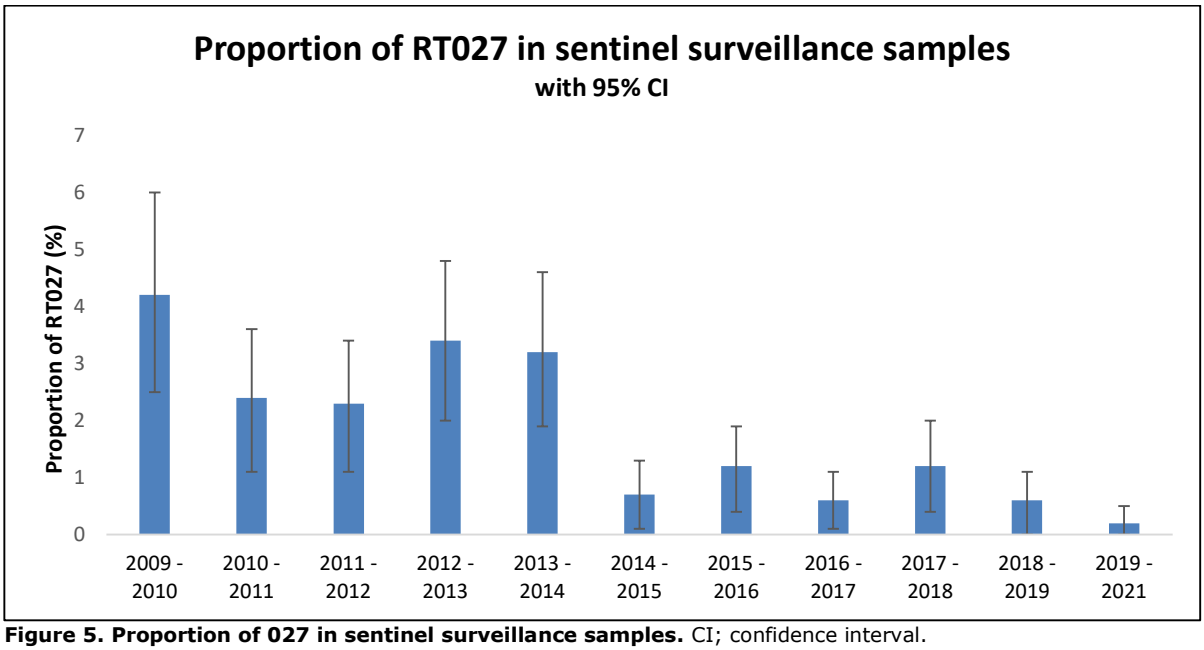


Figure 5. Proportion of 027 in sentinel surveillance samples. CI; confidence interval.

Demographical and clinical data

Demographical and clinical characteristics were collected from 1382 patients included in the sentinel surveillance (Table 1). The median age was 71.0 years (interquartile range 57.5-79.9). Of all patients, 2.7% (n=37) was younger than 18 years old and 63.0% (n=870) was older than 65 years old. Furthermore, 45.0% of the patients had a community-onset of symptoms and 55.0% a healthcare facility-onset of symptoms. Most patients were classified as having non-fatal underlying diseases (61.3%), whereas 21.1% had eventually fatal diseases and 17.6% had quickly fatal diseases. Notably, 17.3% of the CDI patients did not receive a treatment for CDI. A total of 291 patients (21.2%) had severe CDI, defined as bloody diarrhoea and/or diarrhoea with hypovolaemia or hypoalbuminaemia (<20g/L) and/or with fever (T >38.0°C) and leucocytosis (WBC count >15x10^{9}/l), and/or with pseudomembranous colitis. After 30 days, the outcome and course of the disease was known for 753 patients. In total 667 patients (88.6%) had an uncomplicated course of their CDI infection. On the other hand, two patients (0.3%) were admitted to the ICU as a consequence of CDI, one patient (0.1%) needed surgery as a consequence of CDI and 83 patients with CDI (11.0%) died. Fourteen deaths (1.9%) were due or contributable to CDI.

Comparison to previous years

Data from the sentinel surveillance were compared to surveillance data from previous years (Table 2). The CDI incidence was similar as the incidence in previous years. The proportion of patients with severe CDI in May 2019-Jan 2021 was increased compared to May 2018-May 2019 (2019-2021 21.2% (95% CI 19.0-23.4); 2018-2019 16.1% (95% CI 13.7-18.5)). However. the severity of CDI is still lower compared to the start of the surveillance in May 2009-May 2010 with 27.9% (95% CI 23.9-31.9). This may be explained by the decrease in the proportion of ribotype 027. The proportion of patients with a complicated course of CDI was comparable to last year. Furthermore, CDI-related mortality and the overall mortality in CDI patients were comparable to the previous year. The proportion of community-onset CDI cases was 37.0% (95% CI 32.9-41.1) at the start of the surveillance in 2009-2010, which was significantly lower than the proportion in 2019-2021, which was 45.1% (95% CI 42.5-47.7).

The COVID-19 pandemic

To assess the influence of the COVID-19 pandemic on CDI epidemiology, characteristics of CDI patients from the waves of the COVID-19 pandemic were compared to characteristics from the same periods in previous years. For hospitals (n=15 for 2020, n=20 for 2019 and n=18-21 for 2016-2019) that submitted data on monthly patient-days, the overall monthly CDI incidence rates were calculated (figure 6). Data from 2015 were excluded due to low numbers of hospitals with available data on monthly patient-days. In June 2020 (interwave period after first wave), the CDI incidence rate dropped to 2.2 cases per 10,000 patient-days, which was lower compared to 4.1 in 2019 (IRR 0.5; 95%CI 0.4-0.8) as well as 3.1 in 2016-2019 (IRR 0.7; 95%CI 0.5-1.0). This is also observed in the absolute counts: In June there were 36 CDI patients in 2020, 77 in 2019 and an average of 64.5 in 2016-2019.

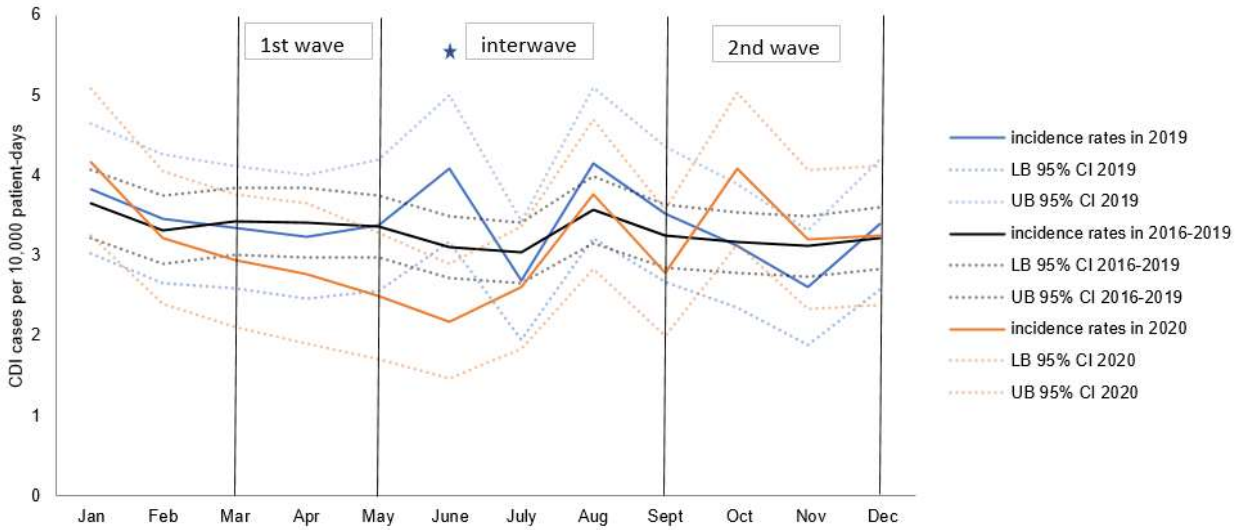


Figure 6. Monthly incidence rates of *C. difficile* infections of hospitals with denominator data available (n=15 for 2020, n=20 for 2019 and n=18-21 for 2016-2019), with 95% confidence intervals. When data on monthly patient-days was not available, data of the previous year was used (except for 2020). Significant differences as assessed by Poisson regression analysis between incidence rates of 2020 compared to 2019 or 2016-2019 were indicated by a blue star. Abbreviations: CDI: *Clostridioides difficile* infection, LB 95% CI: lower boundary of 95% confidence interval, UB 95% CI: upper boundary of 95% confidence interval.

There was no difference in gender between the COVID-19 waves in 2020 and the same periods in 2019 or 2015-2019. Furthermore, the age distribution of CDI patients was compared. The age category 18 to 65 was increased during the first COVID-19 wave (2019: OR 1.8 (95%CI 1.0-3.0); 2015-2019: 1.7 (95%CI 1.1-2.7)), whereas the category 65 to 85 was decreased (OR 0.5 (95%CI 0.3-0.9) and 0.5 (95%CI 0.3-0.8)), compared to both 2019 and 2015-2019. There was no difference for the second wave.

During the second wave in 2020, 25.8% (58/225) of patients had severe CDI, which was higher than the 18.9% (53/281) in the same period in 2019 and 17.9% (232/1293) in 2015-2019, with an OR of 1.5 (95%CI 1.0-2.3) and 1.6 (95%CI 1.1-2.2), respectively. The increased severity of CDI in the second wave was mainly attributed to more dehydration and/or hypoalbuminaemia, which was 13.3% (30/225) in 2020 versus 6.4% (18/281) in 2019 (OR 2.2; 95%CI 1.2-4.1) and 7.7% (100/1,293) in 2015-2019 (OR 1.8; 95%CI 1.2-2.8). There was no difference in severity of CDI for the first wave (15.1% in 2020 vs 16.0% in 2019 with OR 0.9 (95% CI 0.5-1.9) and vs 19.3% in 2015-2019 with OR 0.7 (95% CI 0.4-1.3)).

Notably, the percentage of CDI patients transferred from nursery homes to hospitals was also higher during the second COVID-19 wave in 2020 with 9.3% (21/226) compared to 4.6% (13/281) in 2019 and 5.3% (70/1,326) in 2015-2019 (OR 2.1 (95%CI 1.0-4.3) and 1.8 (95%CI 1.1-3.1), respectively). Furthermore, delayed *C. difficile* diagnostics, defined as a time to *C. difficile* diagnostics from start of symptoms of 8 days or more, was higher during the second COVID-19 wave in 2020. It was observed in 42 of 189 patients (22.2%) during the second wave in 2020, compared to 34 out of 234 patients (14.5%) in the same period in 2019 (OR 2.1; 95%CI 1.0-4.3) and 166 out of 1092 patients (15.2%) in 2015-2019 (OR 1.8; 95%CI 1.1-3.1).

There percentage of patients with a complicated course was higher during the first wave with 24.4% compared to 11.3% in the same period in 2015-2019 (OR 2.5 (95% CI 1.2-5.2)), but not compared to 2019 with 14.1% (2.0; 95% CI 0.9-4.6). This could be explained by the higher mortality, which was 24.4% during the first wave in 2020 compared to 10.4% in 2015-2019 (OR 2.8; 95%CI 1.4-5.7). There was no difference in complicated course during the second wave.

Table 1. Clinical characteristics and outcome of patients participating in the sentinel surveillance (n=1382)

Patient characteristics and outcome	n/n[a]	%
Gender female	698/1381	50.5%
Location of onset CDI		
hospital	681/1381	49.3%
at home	623/1381	45.1%
nursing home	36/1381	2.6%
other health-care facility	40/1381	2.9%
Specialty		
Internal Medicine[1]	689/1379	50.0%
Surgery[2]	139/1379	10.1%
Lung diseases and TB	92/1379	6.7%
Geriatrics	60/1379	4.4%
Gastroenterology	175/1379	12.7%
Cardiology	32/1379	2.3%
ICU	25/1379	1.8%
Neurology	25/1379	1.8%
Pediatrics	35/1379	2.5%
Other or unknown	107/1379	7.8%
Antibiotics prior to CDI	825/1255	65.7%
Recurrence	233/994	23.4%
Treatment		
No treatment	89/513	17.3%
Treatment before admission	5/513	1.0%
Treatment after admission	419/513	81.7%
McCabe score		
Non-fatal	206/336	61.3%
Eventually fatal	71/336	21.1%
Quickly fatal	59/336	17.6%
Severe CDI	291/1370	21.2%
Pseudomembranous colitis	53/1370	3.9%
Hypovolemia or hypo-albuminaemia	126/1370	9.2%
Bloody diarrhoea	67/1370	4.9%
Fever and leucocytosis	113/1370	8.2%
Outcome[3,4]		
Uncomplicated	667/753	88.6%
Surgery needed	1/753	0.1%
ICU admission needed due to CDI	2/753	0.3%
Death, due or contributable to CDI	14/753	1.9%
Death, unrelated to CDI	61/753	8.1%
Death, cause unknown	8/753	1.1%

[1] Internal medicine including hematology, oncology, nephrology

[2] Surgery including orthopaedics and traumatology

[3] Data on complicated course and mortality from between the 2nd of November 2020 until the 10th of January 2021 were excluded due to technical issues with absence of some answer possibilities, indicating missingness at random.

[4] Hospital R: outcome after 3 days instead of after 30 days

Table 2. Data from the sentinel surveillance for the period May 2019 - Jan 2021 compared to the data from preceding years.

Surveillance period (May-May)	2009-2010	2010-2011	2011-2012	2012-2013	2013-2014	2014-2015	2015-2016	2016-2017	2017-2018	2018-2019	2019-2021
Incidence											
Per 10,000 patient-days	2.7	2.8	2.9	2.9	2.9	3.0	3.1	3.0	2.9	3.1	3.2
Location of onset											
Within healthcare facility	63%	73%	69%	63%	64%	59%	58%	59%	55%	54%	55%
At home	37%	27%	31%	37%	36%	41%	42%	41%	45%	46%	45%
Course and outcome[1,2]											
Severe CDI	28%	20%	27%	25%	21%	24%	21%	17%	20%	16%	21%
Uncomplicated course	66%	86%	87%	88%	87%	86%	89%	87%	87%	90%	89%
Deaths contributable to CDI	4%	3%	4%	2%	3%	4%	2%	2%	3%	1%	2%
PCR ribotype 027											
Prevalence	4.2%	2.4%	2.3%	3.4%	3.2%	0.7%	1.2%	0.6%	1.2%	0.6%	0.2%
N reported 027 outbreaks-sentinel surveilance	1	1	0	1	0	0	0	0	0	0	0
N reported 027 outbreaks-ad hoc typing	2	2	1	2	5	1	0	1	0	0	0

[1] Data on complicated course and mortality from between the 2nd of November 2020 until the 10th of January 2021 were excluded due to technical issues with absence of some answer possibilities, indicating missingness at random.
[2] Hospital R: outcome after 3 days instead of after 30 days

Table 3. Number of patients included in the sentinel surveillance per hospital, and incidence data. Period: May 1st 2019 – Jan 1st 2021. The diagnostic test or algorithm used to diagnose CDI is shown per hospital. The incidence per 10,000 patient-days is compared to the results of the previous annual report, demonstrated as an incidence difference.

Hospital	Diagnostic test(s)	Sample selection	% Positive	Monthly PD	Incidence per 10,000 PD May 2019- Jan 2021	Incidence per 10,000 PD May 2018- May 2019	Incidence difference
A	algorithm B	on request only	6.5% (101/1565)	13612	1.91	1.77	0.14
B	algorithm B	3-day rule AND on request	8.5% (77/906)	4824	2.07	2.96	-0.89
C	algorithm A	on request only	5.1% (37/728)	6180	4.53	4.13	0.40
D	algorithm B	on request only	1.6% (33/2084)[3]	7609	2.04	2.54	-0.50
E	algorithm B	other criteria[1]	4.1% (44/1061)	4787	2.30	1.21	1.08
F	NAAT	3-day rule AND on request	8.6% (76/883)	7017	2.49	1.64	0.86
G	algorithm A	on request only	6.0% (183/3060)	13803	3.73	4.09	-0.36
H	algorithm A	3-day rule AND on request	3.4% (120/3548)	9243	2.70	2.47	0.23
I	algorithm A	on request only	10.4% (59/570)	6348	3.07	2.80	0.27
J	NAAT	on request only (formed or unformed)	6.4% (235/3652)	14463	1.94	2.84	-0.91
K	NAAT	on request only	12.9% (186/1443)	10139	4.93	3.76	1.17
L[$]	NAAT and culture of positives	on request only	6.7% (148/2223)	12243	4.12	2.64	1.49
M	NAAT and culture of positives	3-day rule AND on request	7.3% (120/1649)	9915	2.62	3.53	-0.91
N	algorithm B	3-day rule AND on request	10.5% (204/1935)	9526	2.68	3.65	-0.97
O[~]	GDH EIA and NAAT of positives	3-day rule AND on request	6.0% (123/2065)[4]	15042	2.26	3.32	-1.06
P	NAAT*	on request only	9.7% (299/3093)	13494	3.22	3.43	-0.21
Q[£]	algorithm B	on request AND if unformed only	NA	NA	NA	2.72	NA
R[¥]	NAAT and culture of positives	on request AND on indication	7.5% (256/3435)	14063	5.23	5.88	-0.65
S[£]	algorithm B	3-day rule AND on request	NA	NA	NA	4.41	NA
T	NAAT and culture of positives[#]	other criteria[2]	6.2% (431/6919)	16451	4.01	3.83	0.18
U[§]	algorithm A	on request AND if unformed only	6.2% (73/1177)	6867	4.95	NA	NA
V[£]	algorithm A	3-day rule AND on request	NA	NA	NA	5.63	NA
Total			**7.9%**		**3.20**	**3.17**	**0.03**

EIA= enzyme immunoassay; GDH=glutamate dehydrogenase; NA=not available; NAAT=Nucleic Acid Amplification Test; PD=patient-days;
algorithm A: NAAT or GDH EIA- Tox A/B EIA (*ESCMID recommended)*
algorithm B: GDH & Tox A/B EIA (and in some hospitals confirmation with NAAT/TC) *(ESCMID recommended)*
algorithm C: Tox A/B EIA - GDH EIA
[$] One hospital started with the surveillance on 01-07-2020
[~] Combination of a hospital that participated during the whole surveillance period and a hospital of which participation started on 01-02-2020
[£] Data of previous years (data of this year not available), except for number of cases;
[¥] Excluding data from department of rehabilitation, psychiatry and neonatology
[§] Data of 01-10-2019 until 01-01-2021
* Except in the weekend: then GDH and toxin A/B EIA is performed, and confirmation of these with NAAT when not conclusive
[#] Except in the weekend: then GDH and toxin A/B EIA is performed on indication. NAAT was not performed between March 13 until August 3 2020. GDH EIA, Tox A/B EIA and culture of positives were used instead.
[1] All unformed stool samples from inpatients and samples from immunocompromised patients, from patients with acute diarrhea, during increased CDI incidence or on request
[2] All unformed stool samples from in- and outpatients AND for general practitioners on request or based on prescribed antibiotics
[3] Data % positive from 01-01-2019 until 01-01-2021
[4] Only including data from 2020

Table 4. The two most frequently found PCR ribotypes per hospital, isolated amongst patients that were included in the sentinel surveillance. Period: May 1st 2019 – Jan 1st 2021. Ribotype 014/020 are indistinguishable by conventional ribotyping, and ribotype 078/126 can be hardly discriminated.

Hospital	CDI Samples		Sample	*C. difficile isolated*		Most common type			2nd most common type		
	N	%	type	N	%		N	%		N	%
A	52	3.8%	Isolates	41	78.8%	014/020, 078/126	all n=4	9.8% each	015, 118	all n=3	7.3% each
B	20	1.5%	Faeces	18	90.0%	005	5	27.8%	078/126	4	22.2%
C	56	4.1%	Faeces	38	67.9%	078/126	6	15.8%	014/020, 106	all n=4	10.5% each
D	31	2.2%	Isolates	8	25.8%	several[3]	all n=1	12.5% each	-	-	-
E	22	1.6%	Isolates or faeces	3	13.6%	005, 015, 029	all n=1	33.3% each	-	-	-
F	35	2.5%	Isolates	25	71.4%	014/020	7	28.0%	002	3	12.0%
G	103	7.5%	Faeces	71	68.9%	014/020	18	25.4%	several[4]	all n=5	7.0% each
H	50	3.6%	Isolates	44	88.0%	014/020	8	18.2%	002	7	15.9%
I	39	2.8%	Isolates	36	92.3%	014/020	9	25.0%	078/126	6	16.7%
J	56	4.1%	Faeces	53	94.6%	014/020	8	15.1%	001	7	13.2%
K	100	7.3%	Isolates or faeces	66	66.0%	014/020	11	16.7%	002	9	13.6%
L	101	7.3%	Isolates	100	99.0%	078/126	19	19.0%	014/020, 002	all n=12	12.0% each
M	52	3.8%	Isolates	43	82.7%	002	13	30.2%	001	5	11.6%
N	51	3.7%	Faeces	37	72.5%	014/020	8	21.6%	002	5	13.5%
O	68	4.9%	Isolates or faeces	48	70.6%	014/020	11	22.9%	002	6	12.5%
P	87	6.3%	Faeces	74	85.1%	014/020	17	23.0%	005	10	13.5%
Q	44	3.2%	Faeces	35	79.5%	014/020	9	25.7%	005	5	14.3%
R	147	10.7%	Isolates	110	74.8%	014/020	27	24.5%	078/126	11	10.0%
S	33	2.4%	Isolates	32	97.0%	005	5	15.6%	001, 014/020	all n=4	12.5% each
T	132	9.6%	Isolates	104	78.8%	014/020	22	21.2%	005	10	9.6%
U	51	3.7%	Faeces	43	84.3%	002	7	16.3%	014/020	5	11.6%
V	49	3.6%	Faeces	29	59.2%	002	4	13.8%	several[5]	all n=2	6.9% each
Total	**1382**	**100.0%**		**1058**	**76.6%**	**014/020**	**192**	**18.1%**	**002**	**105**	**9.9%**

[1] Participation for only 8 months
[2] Participation for only 15 months
[3] 005, 064, 116, 126, 258, unknown
[4] 002, 005, 015, 078/126
[5] 023, 070, 081, 265, 078/126

Results of the ad hoc typing

Healthcare facilities and laboratories using the Reference Laboratory in suspected outbreaks

In the period between May 1st 2019 and Jan 1st 2021, nine healthcare facilities and laboratories in the Netherlands sent samples to the Reference Laboratory in Leiden for ad hoc typing (Table 5). The samples were sent for other reasons than for sentinel surveillance, such as severe CDI or suspicion of an outbreak. In total, 94 samples were submitted for ad hoc PCR ribotyping.

Ad hoc ribotyping results

C. difficile could be cultured in 89.4% of the 94 submitted samples. The number of submitted isolates/samples and most common PCR ribotypes stratified per facility/laboratory, are demonstrated in table 5. Ribotype 014/020 was the most commonly found PCR ribotype (21.4%), which was also the most common ribotype in May 2018-May 2019. Other frequently found ribotypes were 002 (11.9%), 078/126 (9.5%), 017 (7.1%) and 005 (6.0%). There were no isolates with RT027 in May 2019-Jan 2021, whereas this was still 4.1% (95% CI 0.6-7.6) in May 2018-May 2019. Similar to the sentinel surveillance, the proportion of RT027 appears to decrease in the ad hoc typing service, as it was 15% in 2017-2018, 17% in 2016-2017, 20% in 2015-2016, 14% in 2014-2015, 32% in 2013-2014, 20% in 2012-2013, 15% in 2011-2012, 26% in 2010-2011, and 4% in 2009-2010. The percentage of ribotype 078/126 was similar to last year (2019-2021: 9. 5% with 95% CI 3.2-15.8; 2018-2019: 4.9% with 95% CI 1.1-8.7). Overall, the ribotypes of the ad hoc analysis were rather similar compared to last year. A pie-chart illustrates these findings in comparison to the most common ribotypes of patients included in the sentinel surveillance (Figure 7).

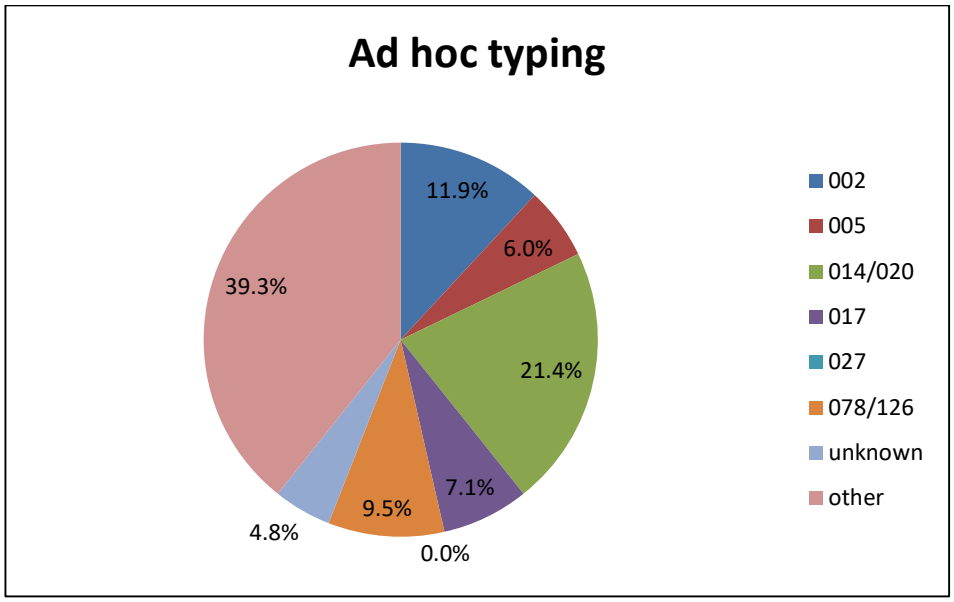
Outbreak investigation

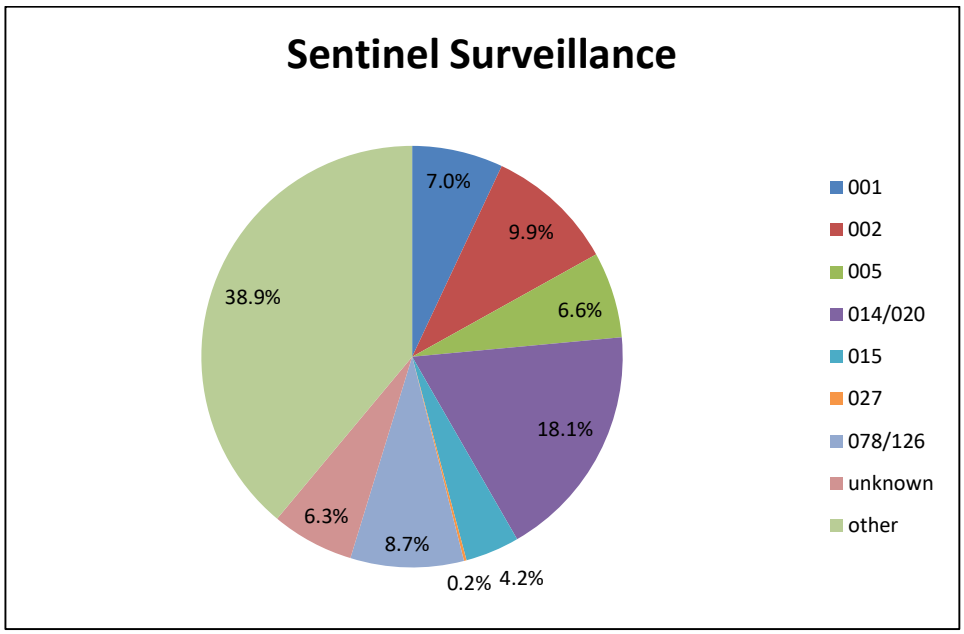
In the period between May 1st 2019 and Jan 1st 2021, no outbreaks of *C. difficile* were reported to the National Reference Laboratory in hospitals not participating in the sentinel surveillance.

Table 5. Results of the ad hoc typing. Period: May 1st 2019 – Jan 1st 2021. Ribotype 014/020 are indistinguishable by conventional ribotyping, and ribotype 078/126 can be hardly discriminated.

Laboratory/Healthcare facility	Samples		Sample	*C. difficile*		Most common ribotypes		
	N	%	type	N	%		N	%
1	2	2.1%	Isolates	1	50.0%	002	1	100.0%
2	1	1.1%	Isolates	1	100.0%	078/126	1	100.0%
3	3	3.2%	Isolates	3	100.0%	005	3	100.0%
4	38	40.4%	Isolates	37	97.4%	014/020	6	16.2%
5	1	1.1%	Faeces	0	0.0%	-	-	-
6	2	2.1%	Faeces	2	100.0%	014/020, 078/126	all n=1	50.0% each
7	41	43.6%	Isolates or faeces	34	82.9%	014/020	9	26.5%
8	5	5.3%	Isolates	5	100.0%	014/020, 017	all n=2	40.0% each
9	1	1.1%	Faeces	1	100.0%	010	1	100.0%
Total	**94**			**84**	**89.4%**	**014/020**	**18**	**21.4%**

Figure 7. Proportions of the 5 most frequently encountered PCR ribotypes and ribotype 027 and 078/126 in the sentinel surveillance data, in comparison to ad hoc typing data. Period: May 1st 2019 – Jan 1st 2021. The category 'other types' consists of 95 different types in the sentinel surveillance data and 21 different PCR-ribotypes in the ad hoc typing data.





Conclusions

The National Reference Laboratory for *C. difficile* ends in 2022

- The Dutch National Reference Laboratory coordinated a sentinel surveillance program with 22 participating acute care hospitals in the Netherlands, and performed molecular characterisation of *C. difficile* in cases of severe CDI or suspected outbreaks ('ad hoc typing service') for other healthcare facilities.
- From 1 January 2022 onwards, the National Reference Laboratory will stop and some of the activities will be continued in a new "Expertise Centre for CDI".

Results of the sentinel surveillance (May 2019- Jan 2021)

- Various CDI diagnostic methods are applied, but an increased number (59.1% of participating hospitals) use optimal diagnostic methods as recommended by ESCMID and ECDC. Although recommended, most hospitals do not test all submitted unformed stool samples of hospitalised patients for CDI. This could lead to an underestimation of the incidence, less recognition of CDI in patients who lack traditional risk factors and might also affect the number of complications and mortality.
- Most laboratories did not often receive test requests for CDI from general practitioners: three often (> 1 per day), nine sometimes (1 or more per week or per month), two sporadically (less than 1 per month), four never and of four laboratories it was unknown whether they received test requests from general practitioners. However, university hospital laboratories rarely or never perform diagnostics for general practitioners.
- A mean incidence rate of 3.20 CDI cases per 10,000 patient-days (varying from 1.91 to 5.23 CDI cases per 10,000 patient-days) was found through sentinel surveillance, similar to last years.
- The disease severity was reported for 1370 out of 1382 patients included in the surveillance; 21.2% had severe CDI. The 30-day outcome was analysed for 753 patients; 88.6% had an uncomplicated course, 0.3% were admitted to the ICU as a consequence of CDI, 0.1% needed surgery as a consequence of CDI and 11.0 % of the patients died within 30 days (n=83). For 14 patients (1.9%) their death was known to be contributable to CDI. CDI severity was higher compared to last year. Other outcomes of CDI were comparable to last year.
  A difference in severity of CDI is observed between the start of the surveillance in May 2009-May 2010 with 27.9%, and May 2019-Jan 2021 with 21.2%. This may be explained by the decrease in the proportion of ribotype 027.
- The proportion of community-onset cases was 45.1%, which was, like previous years, higher compared to the start of the surveillance in May 2009-May 2010 with 37.0%.
- Similar as in May 2018-May 2019, the most frequent encountered PCR ribotype was ribotype 014/020 (18.1%). Unlike May 2018-May 2019, the second most encountered PCR ribotype was 002 (9.9%).
- Ribotype 027 was found in 0.2% of samples (0.6% during May 2018-May 2019).
- No large outbreaks were reported this year.

Results of ad hoc typing (May 2019- Jan 2021)

- Nine healthcare facilities/laboratories sent 94 samples to the Reference Laboratory for ad hoc typing because of suspected outbreaks, severe CDI cases, or for other reasons.
- Ribotype 014/020 was the predominant ribotype (21.4%), followed by ribotype 002 (11.9%) and ribotype 078/126 (9.5%).
- No outbreaks were recognised using PCR-ribotyping and genetic analysis by MLVA or wgMLST.

Effect of COVID-19 pandemic

- There was a decrease in CDI incidence in June 2020, in between the COVID-19 waves.
- There were more patients aged 18 to 65 years and less aged 65 to 85 years during the first COVID-19 wave compared to previous years.
- The increased severity of CDI in the current report, could be explained by the increased CDI severity during the second COVID-19 wave in 2020. This may be caused by delayed diagnostics and decreased referral of patients during the first wave.
- There was an increase in the percentage of patients with a complicated course during the first COVID-19 wave, which could be explained by an increased mortality.

Output of the National Reference Laboratory May 2019-October 2021

Participation of National Reference Laboratory in National and European activities

Granted Tender by ECDC: "Microbiological support to European surveillance of *Clostridium difficile* infections." 2015-2020.

IMI: Combatting Bacterial Resistance in Europe – *Clostridium difficile* Infections (COMBACTE-CDI). 2017-2020

ESCMID: European Society of Clinical Microbiology and Infectious Diseases: 2021 update on the treatment guidance document for *Clostridioides difficile* infection in adults.

Publications May 2019 – October 2021 related to the reference laboratory

Andrés Lasheras S, Martín Burriel I, Aspiroz C, Mainar Jaime RC, Robres P, Sevilla E, Kuijper E, Chirino Trejo M, Bolea R. Incidence and characterization of *Clostridium difficile* in a secondary care hospital in Spain. Rev Esp Enferm Dig. 2019 May;111(5):338-344

Czepiel J, Dróżdż M, Pituch H, Kuijper EJ, Perucki W, Mielimonka A, Goldman S, Wultańska D, Garlicki A, Biesiada G. *Clostridium difficile* infection: review.
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Oral. Amoe Baktash. Application of wgMLST for the epidemiology of CDI.

Clostpath 11TH, 19-22 August 2019, Leiden.

Oral: Ed J, Kuijper. From epidemilogy to National Donor Fecesbank.

Oral 11: Ilse Boekhoud, Bastian Hornung, Eloisa Sevilla, Céline Harmanus, Elisabeth Terveer, Rosa Bolea, Jeroen Corver, E.J. Kuijper, Wiep Klaas Smits. Plasmid-mediated metronidazole resistance in C. difficile.

Poster. Michał Piotrowski, Dorota Wultańska, Piotr Obuch-Woszczatyński, Katarzyna Dzierżanowska-FangratWiep Klaas Smits, Ed J. Kuijper, Hanna M. Pituch.Phenotypic and genotypic analysis of *Clostridium difficile* strains isolated from children hospitalized in a large pediatric center in Poland- retrospectve study.

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Poster 141. Bastian Hornung, E.J. Kuijper, Wiep Klaas Smits.An in silico survey of *Clostridioides difficile* extrachromosomal elements.

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