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Update of Clostridium Difficile Infection Due to PCR Ribotype 027 in Europe, 2008

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Outbreaks of *Clostridium difficile* infections (CDI) with increased severity, high relapse rate and significant mortality have been related to the emergence of a new, hypervirulent *C. difficile* strain in North America and Europe. This emerging strain is referred to as PCR ribotype 027 (Type 027). Since 2005, individual countries have developed surveillance studies about the spread of type 027. *C. difficile* Type 027 has been reported in 16 European countries. It has been responsible for outbreaks in Belgium, Germany, Finland, France, Ireland, Luxembourg, The Netherlands, Switzerland and the United Kingdom (England, Wales, Northern Ireland and Scotland). It has also been detected in Austria, Denmark, Sweden, Norway, Hungary, Poland and Spain. Three countries experienced imported patients with CDI due to Type 027 who acquired the infection abroad. The antimicrobial resistance pattern is changing, and outbreaks due to clindamycin-resistant *ermB* positive Type 027 strains have occurred in three European countries. Ongoing epidemiological surveillance of cases of CDI, with periodic characterisation of the strains involved, is required to detect clustering of cases in time and space and to monitor the emergence of new, highly virulent clones.
Introduction

Since the emergence of a new virulent strain of *Clostridium difficile* characterised as toxinotype III, North American pulsed-field type 1 (NAP1), restriction-endonuclease analysis group type BI and PCR-ribotype 027 (Type 027), multiple outbreaks have been reported in North America and Europe [1-9]. The increased virulence of *C. difficile* Type 027 is thought to be associated with a 1 base pair deletion at position 117 of the tcdC gene which leads to an increased or prolonged production of toxins A and B, and possibly the production of a binary toxin [1-3]. However, these virulence factors are not unique for Type 027 and are also present in other PCR ribotypes.

The first reports of outbreaks of *C. difficile* infections (CDI) due to Type 027 came from Canada, and the province of Quebec was the one affected first and most severely [4]. In the United States, cases of *C. difficile* Type 027 infection have been reported from at least 38 states (http://www.cdc.gov/ncidod/dhqp/id_Cdiff.html), and surveillance of community-acquired CDI has started [10]. By 2007, *C. difficile* Type 027 had been detected in 11 European countries [9]. The present report is an update on the situation in Europe in 2008.

Surveillance efforts

In 2005, the European Study Group for *Clostridium difficile* (ESGC) performed a two-month surveillance study in 38 hospitals from 14 European countries [5]. Unfortunately, only hospital-acquired CDI were studied and no precise information on the severity and outcome was collected. The mean incidence of CDI was 2.45 +/-1.8 cases per 10,000 patient-days. The distribution of PCR ribotypes varied among hospitals and countries. Of 322 toxigenic isolates, 20 (6.2%) belonged to Type 027 and were reported from Ireland, Belgium and The Netherlands. Patients infected with Type 027 were more likely to have a more severe disease, and to have been treated by metronidazole or vancomycin compared to patients infected by another PCR ribotype.

The European Centre for Disease Prevention and Control (ECDC) recognised this emerging new disease and undertook several actions to inform all European Union (EU) Member States. ECDC also offered support for surveillance studies at national and European level. Another pan-European surveillance study is presently being organised, which will collect epidemiological and microbiological data for one month in a selected number of hospitals from all EU Member States in order to estimate the incidence of hospital-acquired as well as community-acquired CDI. The results of this study are expected to be available in 2009.

Austria

In Austria, *C. difficile* Type 027 was reported once in 2006 in a British tourist suffering from pseudomembranous colitis. In May 2008, two cases of CDI due to Type 027 were found in patients who had no travel history in the year before their hospitalisation [11]. Typing patterns of isolates submitted voluntarily since 2006 demonstrate the occurrence of non-027 clusters of CDI cases in Austrian hospitals. The largest cluster affected a tertiary teaching hospital in 2006, where *C. difficile* PCR ribotype 053 represented 10 of 21 CDI cases. CDI is not a reportable disease in Austria. Hospital discharge data indicate a significant increase of CDI during the last years, from 777 cases (54 deaths) in 2003 to 997 cases (80 deaths) in 2004, 1,453 cases (88 deaths) in 2005 and 2,192 cases (150 deaths) in 2006.

Belgium

In Belgium, laboratory-based surveillance of CDI clusters performed by the national reference laboratory at the Université Catholique de Louvain as well as prospective surveillance of CDI incidence in acute care hospitals monitored by the Institute for Public Health were initiated in January 2006. Surveillance of CDI has become a legal obligation since July 2007. In 2007, 896 *C. difficile* isolates were analysed at the reference laboratory.

With 17.6% (158 isolates) Type 027 was the most frequently found type. Other frequently found types were PCR ribotypes 078 and 031, accounting for 6.3% and 5.6% of these isolates, respectively. Overall, the mean (median) incidence of CDI was 1.7 (1.6) cases per 1,000 admissions and 2.07 (1.86) cases per 10,000 hospital days. Sixty-eight percent of these cases occurred more than two days after hospital admission.

Denmark

In April 2006, Statens Serum Institut encouraged the Danish departments of clinical microbiology to report *C. difficile* cases on a continuous basis and to forward isolates for characterisation in cases of severe disease or in outbreak situations. In a retrospective survey covering a county in Region South Denmark, a cluster of eight patients with *C. difficile* Type 027 was detected. The isolates were recovered from 22 faecal samples that had been collected between November 2006 and March 2007. All eight cases were hospitalised in two hospitals in the region. Subsequently, active surveillance was initiated in the same region for the period June-August 2007, which resulted in five additional Type 027 cases among 22 *C. difficile* isolates tested. Interestingly, all 13 isolates were resistant to newer fluoroquinolones and cephalosporins, but susceptible to erythromycin and clindamycin.

Finland

The first case of *C. difficile* Type 027-associated disease was detected in Finland in October 2007 [12]. Since then the National Public Health Institute (Kansanterveyslaitos; KTL) has intensified surveillance and control of CDI. A few additional cases caused by Type 027 were detected retrospectively, indicating that this strain had previously been circulating in Finland. The Finnish Hospital Infection Programme (SIRO) prepared a protocol for CDI surveillance to detect severe cases and epidemics caused by *C. difficile*. Molecular methods for rapid detection of *C. difficile* Type 027 were set up at two clinical, university-affiliated laboratories in Helsinki and Turku, and genotyping methods for molecular epidemiology of *C. difficile* were set up at KTL.

During the five-month-period from mid-October 2007 to mid-March 2008, isolates of *C. difficile* Type 027 were reported from four of the nine health care districts that had sent the isolates to KTL, and originated from over 20 different health care facilities – most of them providing primary or long term care – located in southern and south-western Finland. Of the 268 isolates, 131 (49%) belonged to Type 027. The remaining isolates were distributed among more than 30 different PCR ribotypes.

France

In France, the CDI surveillance is based on the mandatory notification of severe cases or outbreaks of CDI to local health departments, regional infection control coordinating centres and the National Institute for Public Health (Institut de Veille Sanitaire;
Laboratories are encouraged to send the isolates from notified cases to a network of six French reference laboratories for *C. difficile*.

In April 2006, the first cluster of *C. difficile* Type 027 was reported in Northern France. From January 2006 to December 2007, 214 health care facilities reported at least one severe case or outbreak of CDI and a total of 1,247 cases. Sixty-four health care facilities (29 in 2006 and 35 in 2007, with no overlap between these 64) were affected by Type 027. Most cases originated from healthcare facilities in the Nord Pas-de-Calais region, but in 2007, small clusters of *C. difficile* Type 027 were reported from three other French regions, Picardie, Rhône-Alpes and Lorraine. Among the 1,227 isolates (511 in 2006 and 716 in 2007) sent for typing, 337 (27.5%) were identified as Type 027 (212 in 2006, i.e. 41.5% of the typed isolates, and 125 in 2007, i.e. 17.4% of the typed isolates). The large majority of strains were resistant to erythromycin and moxifloxacin, but susceptible to clindamycin. However, one hospital in Picardie reported an outbreak associated with a clindamycin-resistant strain that tested positive for the ermB gene encoding the macrolide-lincosamide-streptogramin B (MLSB) phenotype.

Unfortunately, no data are available on the occurrence of other PCR ribotypes.

**Germany**

Since October 2007, it is mandatory to report severe cases of CDI to the local authorities. Patient-based notifications are done by the physician treating the patient. Severe CDI cases are defined as cases which necessitate readmission to a healthcare facility due to the relapse of CDI, admission to an intensive care unit for treatment of CDI or its complications, surgery (colectomy) for toxic megacolon, perforation or refractory colitis, or lead to death within 30 days after diagnosis of CDI, if CDI is either the primary or a contributive cause to death. This mandatory surveillance was implemented shortly after the first outbreak of *C. difficile* Type 027 was detected in the region of Trier, Rhineland-Palatine in September 2007. To date, five of 16 Federal States (Länder), all of which are located in the south-west of Germany, have reported cases of CDI due to Type 027 [13,14].

**Hungary (not included in the table)**

A recently completed surveillance study in three different parts of Hungary revealed one isolate of Type 027 among 150 *C. difficile* isolates collected. The patient had systemic lupus erythematosus and developed severe CDI after antibiotic treatment for pneumonia in a hospital in Budapest.

**Ireland**

After the first report of *C. difficile* Type 027 in Ireland in 2007, this type was identified in six additional healthcare settings [15,16]. To date, more than 100 *C. difficile* Type 027 isolates from Ireland have been characterised by toxotyping and 16-23S PCR ribotyping [15]. Isolates from two healthcare settings were susceptible to clindamycin (n=11: MIC90=4 mg/l). However, clindamycin-resistant Type 027 isolates (n=96, MIC90>256 mg/l) were identified in the five other healthcare institutions. All clindamycin-resistant Type 27 isolates tested positive for the ermB gene. Multiple locus variable number tandem repeat (MLVA) typing could clearly differentiate between clindamycin-resistant and -susceptible isolates from the same geographical region and sub-grouped them into two distinct clusters, with all isolates from the clindamycin-resistant cluster being closely related [16].

CDI has become a notifiable disease in the Republic of Ireland since May 2008 under ‘acute infectious gastroenteritis’ using the case definition by ESGCD and ECDC. Only new cases will be reported, and this will enable data to be collected on the national level, but not on hospital-level. There are moves to make CDI notifiable in its own right to enable the collection of enhanced surveillance data (e.g. on origin and onset of CDI). National guidelines on surveillance, diagnosis and management of *C. difficile* have been published in May 2008 [17].

**Luxembourg**

During the period between October 2006 (start of CDI surveillance in Luxembourg) and February 2008, 96 (26%) of 368 submitted *C. difficile* strains were PCR ribotyped as Type 027. Type 027 was the type found most frequently, followed by types 001 and 106, but confirmation for the latter two is pending. The isolates came from all 10 hospitals in Luxembourg. The situation is ongoing and the total number of *C. difficile* isolates is now exceeding the number of salmonella and campylobacter isolates.

The median age significantly differed between patients with Type 027 (74 years) and patients with other ribotypes (59 years) (p=0.001). The mortality rate of CDI due to Type 027 within one month and within three months of isolate referral was 14.8% and 21.0%, respectively. In a logistic regression model, one-month mortality of CDI was significantly associated with age over 70 years (p<0.0001), but not with gender (p=0.66) or PCR ribotype (p=0.14).

**Netherlands**

Since October 2005, the Centre for Infectious Disease Control (Cib) at the National Institute for Public Health and the Environment (Rijks Instituut voor Volksgezondheid en Milieu; RIVM) and the reference laboratory for *C. difficile* at Leiden University Medical Center have encouraged microbiologists to send *C. difficile* isolates from patients with a severe course of CDI, or when an increased incidence of CDI was noticed. During the surveillance period from 2005 to 2007, Type 027 was reported from an increasing number of healthcare facilities in an endemic form or in outbreaks. At the end of 2007, 35 healthcare facilities have been affected, compared to 22 healthcare facilities until the end of 2006 [8]. During the surveillance period of 2006/2007, five outbreaks with Type 027 occurred, compared to 11 outbreaks in 2005/2006. One hospital was affected by an outbreak caused by both Type 027 and Type 017.

Comparison of clinical data of patients with CDI due to Type 027 (n=128) and other types (n=443) showed that CDI due to Type 027 was associated with older age, use of cephalosporins (mainly second generation) and fluoroquinolones (mainly ciprofloxacin). Patients with Type 027 CDI had more relapses and a more severe disease with a higher overall and attributable mortality [8]. *C. difficile* Type 027 was significantly more often acquired at a health care institution. Other frequently isolated PCR ribotypes in The Netherlands were types 014, 001 and 078.

**Norway**

In December 2007, the first two cases of CDI due to Type 027 in Norway were reported from a university hospital in Oslo [18].
Surveillance and infection control measures did not reveal other Type 027 isolates at this hospital. In February 2008, a third case of CDI due to Type 027 was detected at a nursing home in Oslo.

Since January 2008, the Department of Infection Prevention in cooperation with the Institute of Microbiology, both at Rikshospitalet University Hospital, Oslo, have performed genotypic characterisation of *C. difficile*. The most frequently found PCR ribotype is Type 014. Unfortunately, most medical microbiology laboratories in Norway do not cultivate *C. difficile*. As a consequence, the distribution of PCR ribotypes in Norway remains unknown.

**Poland**

No systematic CDI surveillance has yet been developed in Poland. Between 2005 and 2007, a surveillance study was performed in four hospitals in the Mazovia region. Of 400 *C. difficile* isolates, one isolate belonged to Type 027. As determined by E-tests, the isolate was highly resistant to fluoroquinolones (ciprofloxacin, gatifloxacin and moxifloxacin, MIC≥32 mg/l) and erythromycin (MIC≥256 mg/l), but susceptible to clindamycin (MIC=6 mg/l), metronidazole (MIC=0.38 mg/l) and vancomycin (MIC=0.75 mg/l). The most frequent PCR ribotype was Type 017, which accounted for approximately 40% of the *C. difficile* isolates studied.

**Spain**

Spain does not have a national surveillance programme to investigate cases of CDI or an official reference laboratory where hospitals could send *C. difficile* isolates for further characterisation. A surveillance study performed between January and June 2007 at a 1,750-bed, tertiary care hospital in Madrid revealed two cases of severe CDI due to Type 027. The index case was a Spanish patient admitted to the intensive care unit, who was transferred from a hospital in the United Kingdom. The other patient was a laboratory technician working with *C. difficile* isolates, who developed CDI shortly after antibiotic treatment. In this study, a non-specified PCR ribotype containing the genes for toxins A and B but not for the binary toxin, was detected in 103 of 388 typed *C. difficile* isolates (26.5%, 81 patients).

Since the *C. difficile* Type 027 has the binary toxin genes, testing for the presence of these genes is performed for all *C. difficile* isolates. Binary toxin-positive strains are subsequently ribotyped. In contrast to previous studies performed in this hospital, there was an increase of non-027 *C. difficile* containing the genes for toxins A and B and the genes for the binary toxin (13% of the total number of isolates studied). The PCR ribotype pattern of the binary toxin positive isolates probably corresponds to Type 078.

**Sweden**

Three sporadic ‘historical’ moxifloxacin-susceptible isolates of *C. difficile* Type 027 were found among 1,325 isolates collected between 1997 and 2001 in Sweden. In September 2006, the Swedish Institute for Infectious Disease Control (Smittskyddsinstitutet; SMI) alerted microbiologists and clinicians about *C. difficile* Type 027 and laboratories were encouraged to send *C. difficile* isolates to SMI for microbiological characterisation for patients with a severe course of CDI or when an increased CDI incidence was noticed.

Since epidemic Type 027 isolates have uniformly been moxifloxacin-resistant, a systematic screening of *C. difficile* isolates for moxifloxacin resistance was initiated during 2007 in four hospitals in Stockholm. In February 2008, this screening was extended to include all major hospitals in Sweden. Preliminary results indicate only one case of moxifloxacin-resistant Type 027 (found in May 2008), but there is currently no indication of outbreaks due to *C. difficile* Type 027. The most frequently PCR ribotypes isolated in Sweden are Types 012 and 014.

**Switzerland**

In Switzerland, the first outbreak of *C. difficile* Type 027 was observed in a geriatric hospital in Basel in 2006 [19]. The index case was an 82-year-old female patient and the outbreak involved 15 other patients between October 2006 and May 2007. It is likely that the index patient acquired *C. difficile* Type 027 during a hospital stay in a foreign country. The median age of the 16 patients was 83.5 years (interquartile range: 79–92 years). A severe to moderate course of CDI was reported in seven (44%) of the patients and crude mortality was 19% (three deaths). All isolates were highly resistant to moxifloxacin (MIC>32 mg/l), erythromycin (MIC>256 mg/l) and clindamycin (MIC>256 mg/l). MLVA typing revealed one cluster of genetically highly related (STRD<2) clindamycin-resistant Type 027 isolates which differed from the clindamycin-susceptible Type 027 control isolates and also from clindamycin-resistant isolates from Ireland.

**United Kingdom (UK)**

In England and Wales, mandator surveillance of CDI in patients over 65 years has been included in the healthcare-associated infection surveillance system for acute trusts [20]. This mandatory surveillance programme is operated by the Health Protection Agency (HPA) on behalf of the Department of Health. Through its network of regional laboratories in collaboration with the *C. difficile* Ribotyping Network for England (CDRNE) and the Anaerobe Reference Laboratory (ARL) in Cardiff, the HPA further obtains *C. difficile* isolates from symptomatic patients in a structured, but random sampling scheme. In England, 110 out of 145 hospitals (76%) investigated between April 2007 and February 2008 showed the presence of *C. difficile* Type 027. Of 2,084 *C. difficile* isolates, 42% were typed as Type 027, 19% as Type 016 and 10% as Type 011. In Wales, 10 out of 16 investigated hospitals showed the presence of *C. difficile* Type 027.

In Scotland all diagnostic laboratories have been requested since September 2006 to submit *C. difficile* isolates to a UK reference laboratory in the case of severe CDI or outbreaks. The data are published quarterly [21]. Additionally, isolates from local research projects have also been submitted for ribotyping, which means that some hospitals/regions are over-represented in this collection of isolates. A total of 20 cases of *C. difficile* Type 027 were identified in Scotland in the period from September 2006 to April 2008. Among these were an outbreak with five cases in one hospital in the West of Scotland and an outbreak with three cases in a hospital in the North East of Scotland. In total, Type 027 has been detected in nine acute care hospitals in five different geographical regions of Scotland. One case was reported from a nursing home.

Until recently, *C. difficile* Type 027 was not a common PCR ribotype in Scotland. With the two recent outbreaks the frequency of 027 has reached 5.7%. Since 2006, the most frequent PCR ribotypes in Scotland have consistently been type 106 (55% of *C. difficile* isolates) and type 001 (21%). Four isolates of the new emerging ribotype 078 have been identified in Scotland as well.

In Northern Ireland, a survey was undertaken between September and December 2006, and 60 samples (4.0% of the annual total of *C. difficile* isolates) were examined.
<table>
<thead>
<tr>
<th>Country</th>
<th>Survey period</th>
<th>Population* (million inhabitants, 2006)</th>
<th>No. of hospitals* (2006, if not otherwise mentioned)</th>
<th>No. of hospital beds* (2006, if not otherwise mentioned)</th>
<th>No. of positive hospitals / No. of hospitals investigated for Type 027 (%)</th>
<th>No. of nursing homes positive for Type 027</th>
<th>No. of Type 027 isolates / Total no. of isolates tested (%)**</th>
<th>Attributable mortality of C. difficile infections</th>
<th>Updates available at</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>2006</td>
<td>8.3</td>
<td>total: 264; acute care: 183</td>
<td>total: 63,35% acute care: 52,8%</td>
<td>1 / 43 (2%)</td>
<td>n.a.</td>
<td>1 / 1,004 (&lt;1%)</td>
<td>n.a.</td>
<td><a href="http://webribo.ages.at">http://webribo.ages.at</a></td>
</tr>
<tr>
<td>Belgium</td>
<td>2007</td>
<td>10.4</td>
<td>total: 215; acute care: 146</td>
<td>total: 55,156 acute care: 49,435</td>
<td>32 / 74 (43%)</td>
<td>n.a.</td>
<td>158 / 896 (18%)</td>
<td>see text</td>
<td><a href="http://www.belgianinfectioncontrol.society.be/">http://www.belgianinfectioncontrol.society.be/</a></td>
</tr>
<tr>
<td>Germany</td>
<td>Oct. 2007–Apr. 2008</td>
<td>82.7</td>
<td>total: 3,35% acute care: 2,104</td>
<td>total: 683,48% acute care: 510,767</td>
<td>13 / n.a.</td>
<td>1</td>
<td>44 / n.a.</td>
<td>2.3%</td>
<td><a href="http://www.rki.de">http://www.rki.de</a> &gt; Infektionskrankheiten A-Z &gt; Clostridium difficile</td>
</tr>
<tr>
<td>Poland</td>
<td>2005</td>
<td>38.1</td>
<td>total: 835 (2005); acute care: n.a.</td>
<td>total: 199,789; acute care: 178,074 (2002)</td>
<td>1 / 4</td>
<td>n.a.</td>
<td>1 / 400 (&lt;1%)</td>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>Jan 2007–July 2007</td>
<td>44.4</td>
<td>total: 788</td>
<td>total: 159,671</td>
<td>1 / 1</td>
<td>n.a.</td>
<td>4 / 388 (unknown)</td>
<td></td>
<td></td>
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<tr>
<td>United Kingdom</td>
<td></td>
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</tr>
<tr>
<td>England</td>
<td>2006–2007</td>
<td>50.7</td>
<td>total: 170 trusts (acute care)</td>
<td>total: 126,976 (acute care, 2005/2007)</td>
<td>110 / 346 (76%)</td>
<td>879 / 2,004 (42%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern Ireland</td>
<td>2006 (sept–dec)</td>
<td>1.7</td>
<td>total: 31 trusts (acute care)</td>
<td>total: 6,220</td>
<td>n.a.</td>
<td>n.a.</td>
<td>0 / 50 (unknown)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** Number of C. difficile isolates may differ from number of patients mentioned in the text.
† n.a.: not available.
‡ Total no. isolates screened for moxifloxacin resistance (n=238). PCR ribotyping was only performed on moxifloxacin-resistant isolates (n=60).
difficile reports) were ribotyped: the most common ribotypes were 001 (35%), 106 (11.6%) and 078 (8.3%). Ribotype 027 was not identified in this small sample. The first report of ribotype 027 in Northern Ireland related to a specimen in mid-June 2007. Since then there has been a large hospital outbreak associated with ribotype 027 (57 reports to date).

An enhanced ribotyping surveillance programme has recently been established: 59 specimens were ribotyped, of which 35% were Type 078, 25% were Type 001 and 8% were Type 014/20. The sample contained two reports of Type 027 (3%). Compared with the earlier survey in 2006 there has been a marked increase in ribotype 078 and a decrease in ribotype 001. Further investigations are underway to analyse this change in ribotype incidence.

Conclusion

As of June 2008, C. difficile Type 027 has been reported from healthcare facilities in 16 European countries (Figure, Table). Among those, nine countries have reported outbreaks and seven countries have reported only sporadic cases. Because of the lack of national surveillance programmes in many countries, it is at present impossible to estimate the incidence of C. difficile Type 027 in Europe. A new, emerging Type 078 strain, with similar mechanisms for the hyper-production of toxins as Type 027, is increasingly circulating in Europe since the distribution of these ribotypes varies greatly among European countries and over time.

The occurrence of outbreaks due to clindamycin-resistant Type 027 isolates in three European countries is worrying. Clindamycin has been considered as a ‘protective’ antibiotic with regards to the development of CDI due to Type 027 [8]. However, resistance to clindamycin may increase the risk of CDI in patients receiving this agent and its use may be an important factor contributing to its persistence and spread. In addition, the report of erythromycin-susceptible and clindamycin-susceptible Type 027 isolates in Germany and Denmark indicates that antimicrobial resistance patterns are very dynamic and can no longer be used to identify C. difficile Type 027.

All European countries should now be aware about CDI in healthcare facilities, and specifically about C. difficile Type 027. Surveillance studies should be performed with uniform definitions, as proposed by ECDC [1]. These surveillance studies should not only focus on C. difficile Type 027, but include all major PCR ribotypes circulating in Europe since the distribution of these ribotypes varies greatly among European countries and over time.

References


