

## Emergence of *Clostridium difficile*-associated diarrhoea in Europe.

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*Clostridium difficile* is an anaerobic bacterium, widely distributed in soil and in the intestinal tracts of animals. Its vegetative cells are capable of forming spores, which confer resistance to heating, drying and chemical agents, including disinfectants. *C. difficile* was identified as the cause of pseudomembranous colitis and its milder form, *C. difficile*-associated diarrhoea, in the 1970s. The spectrum of disease ranges from asymptomatic carriage to a fulminant, relapsing and potentially fatal colitis. *C. difficile*, with more than 150 PCR ribotypes and 24 toxinotypes, has a pathogenicity locus (PaLoc) with genes encoding enterotoxin A (tcdA) and cytotoxin B (tcdB). Genes for the binary toxin are located outside the PaLoc, but the role of this toxin is unclear. *C. difficile* also appears to be an important cause of enteric disease in a variety of animal species, including horses, dogs, cats, birds, rodents, and especially neonatal pigs, suggesting that animals may serve as a reservoir for human pathogens (1).

Recent outbreaks of CDAD with increased severity, high relapse rates and significant mortality have been related to the emergence of a new, hypervirulent *C. difficile* strain in 2003 in Canada and the United States (2–5). The predominant strain is referred to as North American pulsed-field type 1 (NAP1), PCR ribotype 027, and group BI by restriction endonuclease analysis. Strain NAP1 contains an 18-base pair (bp) and a deletion at 117 of the tcdC gene. This strain has been associated with the in-vitro production of toxins A and B in quantities 16 and 23 times, respectively, greater than production by control strains. In addition, NAP1 also produces a binary toxin, encoded by the cdtA gene (the enzymic component) and the cdtB gene (the binding component). The extent to which this toxin contributes to the pathogenicity of *C. difficile*, however, is unknown.

*C. difficile* PCR ribotype 027 (and 001) sporulate more frequently than other strains, which may contribute to survival and spread (6). The clonality of *C. difficile* PCR 027 is currently a topic of research. PCR ribotype 027 exhibits at least two pulsed field gel electrophoresis (PFGE) patterns with 94% similarity: north American PFGE types 1a and 1b (NAP1a and NAP1b). As already demonstrated for PCR ribotype 001, other typing techniques (DNA fingerprinting, rapid enzymatic assay, arbitrarily primed PCR) may reveal additional subgroups. Recent publications also demonstrate that multilocus variable-number tandem-repeat analysis can differentiate various subtypes and clusters of Type 027 (7,8).

The Centers for Disease Control and Prevention (CDC) reported the strain to be associated with high rates of morbidity and mortality during outbreaks in hospitals in at least 38 US states (<http://www.cdc.gov/ncidod/dhqp/index.html>), December 2007. Soon after the finding

of PCR type 027 in north America, reports confirmed the presence of this new emerging strain in England, Scotland (one patient), Ireland, Belgium, France, Austria (imported patient), Spain (imported patient), Luxembourg (unpublished), Poland, and the Netherlands (9-14). Very recently, PCR type 027 has also been found in Switzerland (submitted), Denmark (unpublished), Germany, Finland and Norway (submitted) (15-17). The strain was also found in Japan, but surprisingly was susceptible to fluoroquinolones (18). This strain revealed more similarities with the historical PCR ribotype 027 isolates than with the currently circulating strains.

Information on the incidence of CDAD in Europe is available from two European surveys which were performed by the ESCMID Study Group for *C. difficile* (ESGCD). The aims of ESGCD are to determine the prevalence of nosocomial *C. difficile* infections in European hospitals, to see if it is feasible to adopt a standardised PCR ribotyping method, to compare the types of *C. difficile* prevalent in European hospitals, to provide surveillance on the antimicrobial susceptibility of European strains of *C. difficile*, and to draw up European guidelines on the prevention, diagnosis, treatment and surveillance of *C. difficile* infections. The first survey involved 212 hospitals in the UK, France, Belgium, Denmark, Germany, Italy and Spain in 2002 (19). The incidence was 11 per 10 000 admissions. In contrast, data from studies in the USA showed that the incidence among hospitalized patients is much higher, ranging from 10 to 200 per 10,000 admissions. In 2005, a second European surveillance study was performed in 38 hospitals from 14 different European countries (20). The data from 38 hospitals in 14 different countries indicate wide variations in the incidence of CDAD, ranging from 0.13 to 7.1 per 10,000 patient-days (mean 2.45 per 10,000 patients days). The incidence was higher in countries that experienced recent *C. difficile* 027 outbreaks i.e. The Netherlands, Belgium or France. The prevalence of the 027 epidemic strain was 5.7%.

In **England**, a mandatory surveillance programme of CDAD in people aged 65 years and over has been included in the healthcare-associated infection surveillance system for acute hospital trusts since January 2004. The results of a mandatory surveillance of CDAD in individuals over 65 years of age revealed 51 690 CDAD cases in 2005, a 17.2% increase over 2004. In contrast to previous years, non-001 types predominated, specifically types 106 and 027. Type 106 has not been recognized elsewhere, and data on its severity or relapse rate are currently unknown. Some 55,681 cases of CDAD were reported in 2006. This represents an 8% increase in CDAD cases from 2005 to 2006. The number of death certificates in England and Wales that mentioned *C. difficile* increased from 1214 in 2001 to 3807 in 2004. Between 2004 and 2005 the number of deaths involving *C. difficile* increased by 69% (<http://www.statistics.gov.uk/cci/nugget.asp?id=1735>). Most of the deaths were in the elderly population. Epidemiological data are collected quarterly from each of the 169 acute National Health Service (NHS) trusts that treat adult patients and yearly reports are produced by the HPA]. Through its network of regional laboratories in collaboration with the Anaerobe Reference Laboratory (ARL) in Cardiff, the HPA obtained further isolates of *C. difficile* from symptomatic patients in a structured but random sampling scheme. It revealed a widespread dissemination of type 027 to 89 locations in England (11,21).



Figure. Spread of *C. difficile* PCR ribotype 027 in Europe (December, 2007)

**Denmark.** The patient, a woman born in 1922, was admitted to a small county hospital in Brørup (Jutland) on the 25th of October 2006 with pneumonia. She was initially given penicillin and subsequently a fluoroquinolone after which she developed bloody diarrhoea. Type 027 was isolated from the feces sample (K.E.P Olsen, SSI, Copenhagen, Denmark).

**Ireland.** At least 7 hospitals are affected with more than 100 isolates of *C. difficile* 027. Two different clones are circulating; one clindamycin resistant clone and another clindamycin susceptible clone (D. Drudy, submitted for publication).

**Luxembourg.** At least 4 hospitals has been found with outbreaks due to Type 027 and each week several new cases are reported.

In October 2005, the National Institute for Public Health and the Environment (RIVM) in the **Netherlands** published specific CDAD ribotype 027 guidelines for infection control and treatment to be used by hospitals and nursing homes in response to two outbreaks in the Netherlands (10). Diagnostic facilities were increased and made accessible for hospital microbiologists. All laboratories were recommended to culture *C. difficile* from toxin positive faeces samples and to store the isolates. Microbiologists were requested to send strains to the national Reference laboratory from patients with a severe course of CDAD or when an

increased incidence of CDAD was noticed. A National Reference Laboratory for *C. difficile* was established at the Department of Medical Microbiology at the Leiden University Medical Center. Strains were characterised by PCR ribotyping, toxinotyping, presence of toxin genes and antimicrobial susceptibility (9, 10). Until December 2007, ribotype 027 has been detected in 26 of 97 Dutch hospitals in total. In 13 of the affected hospitals, the introduction of 027 caused an increased incidence of CDAD, two of which occurred since December, 2006. Ribotype 027 has also been detected in ten nursing homes. In eight of 11 hospitals where ribotype 027 was detected in 2005 or 2006 and an outbreak occurred, no ribotype 027 has been detected since April, 2007. Two hospitals that had the epidemic well under control for a long time were faced with a new increase in incidence.

Recommendations for diagnosis, early warning and surveillance of CDAD in **France** were issued by the French Institute for Public Health (InVS) and the national reference laboratory for *C. difficile* (Hôpital Saint-Antoine, Paris) in May 2006. Hospitals and nursing homes were requested to notify severe cases or clusters of CDAD, which were systematically investigated by local health departments and regional infection control coordinating centres. Culture of faeces was promoted as the diagnostic method of choice for such cases, and a network of six regional laboratories was set up in order to facilitate characterisation of *C. difficile* strains. The Ministry of Health disseminated recommendations for CDAD prevention and control to all hospitals and nursing homes in September 2006. Until May 2007, more than 40 hospitals have been affected by *C. difficile* type 027, mainly located in the Northern part of France. The reference laboratory has typed more than 280 strains as 027.

One case of *C. difficile* 027 was identified in **Scotland** in 2006 by the UK national reference laboratory in Cardiff. A research study in Western Scotland examined 102 additional strains obtained from nine hospitals from 2006 to 2007. None of these were ribotype 027. Mandatory surveillance in line with the English system has been initiated in Scotland in 2006. Data on the incidence of *C. difficile* 027 in people aged 65 years or older are being collected in healthcare institutions in Scotland and will be published in the public domain by the end of 2007.

In **Belgium**, the Scientific Institute of Public Health (IPH) and the national reference laboratory (Université catholique de Louvain) set up a laboratory-based surveillance of CDAD clusters in January 2006. Laboratories are requested to send in strains, when two or more CDAD cases occur in the same department within a period of one month. In parallel, a prospective surveillance of CDAD incidence was set up in Belgian acute care hospitals in collaboration with the Belgian Infection Control Society (BICS). Hospitals report clinical and risk factor data on all CDAD cases as well as denominator data on a web-based data entry form during a six month surveillance period. Hospitals are also requested to send strains of five consecutive CDAD patients to the reference laboratory for species confirmation, detection of the *tcdC* deletion and the binary toxin, toxinotyping, PCR ribotyping and determination of antimicrobial susceptibility. National guidelines for prevention and control of CDAD in hospitals and nursing homes were issued by the BICS in June 2006. Of 78 hospitals in Belgium, 38 (49%) have been affected by *C. difficile* type 027 and the National Reference laboratory typed over 200 strains as type 027.

In late September 2007, the local health authorities in **Trier**, Rhineland-Palatine, in south-western **Germany**, were informed of four cases with a severe course of CDAD on several wards in a local hospital (15). The strains could be further characterised as PCR ribotype 027, toxinotype III. The strain demonstrated resistance to erythromycin and moxifloxacin, among other antimicrobials, but was susceptible to clindamycin, thereby exhibiting a similar profile to that seen for the highly virulent strains that have caused outbreaks in North

America and several European countries. As of November 2007, eight confirmed and 28 probable cases of *C. difficile* PCR ribotype 027 were identified in six hospitals in the region of Trier. The cases include 16 male and 20 female patients. The mean age was 74 years. Six patients died due to a cause attributable directly or indirectly to the CDAD. Two small clusters comprising a total of six cases were identified in one hospital. In March 2007, a 76-year-old man was admitted to a hospital in **Stuttgart, southern Germany**, where he developed CDAD due to of *C. difficile* PCR ribotype. There was no indication of an outbreak situation. This report indicates that this strain was already present in Germany in March 2007 (16).

On 18 October 2007, the first case of *Clostridium difficile* PCR ribotype 027-associated disease was detected in **Finland** (17). The strain was isolated from a middle-aged male patient who died from a severe pseudomembranous colitis. Two additional cases were detected in a retrospective survey performed in the Helsinki and Uusimaa healthcare district in southern Finland between 2 May and 23 June 2007. None of the three cases of *C. difficile* PCR ribotype 027-associated disease had connections with foreign countries and no connections between the cases could be identified. The attending physicians have been informed and further investigations are ongoing to identify potential additional cases.

At 14 December, the first case of CDAD due to PCR ribotype 027 has been found in an university hospital in **Norway**. The patient was treated with mecillinam for an urinary tract infection and subsequently developed severe CDAD with perforation of the bowel.

**Imported cases.** The increase of travel has a large influence on the rapid spread of infectious diseases. Currently, reports from 3 European countries indicate that *C. difficile* also crosses country borders by the transfer of infected patients. In 2006, in a local hospital in Tyrol, west **Austria**, a 69 year old British woman was admitted to hospital with a five day history of nausea, watery diarrhoea and lower abdominal pain (13). She was nursed in isolation and CDAD due to PCR ribotype 027 was diagnosed. No further spread in the hospital occurred. A cluster of 16 patients with CDAD due to clindamycin-resistant *Clostridium difficile* PCR-ribotype 027 in **Switzerland** most likely was imported from abroad (L. Fenner, A. Widmer, A. Stranden et al. submitted for publication). The index case was a 82-year old female patient who had been hospitalized in Spain before a new admission in Switzerland. The hospital in Spain had many tourists who became ill during their holidays. In **Spain**, a case of CDAD due to PCR ribotype 027 has been diagnosed in a patient with alveolar proteinosis transferred to Madrid from the United Kingdom and admitted to the adult Intensive Care Unit. She developed an episode of CDAD after the use of systemic antibiotics for the treatment of ventilator-associated pneumonia (prof. dr. Emilio Bouza, personal communication).

**Conclusion.** Until December 2007, 10 European countries encountered outbreaks and 7 additional countries had endemic cases of CDAD due to *C. difficile* PCR ribotype 027. This new emerging PCR ribotype is still spreading but an increasing number of countries have provided diagnostic facilities and national guidelines to recognize this type in an early stage and to combat further spreading.

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