

UPDATE OF *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DISEASE DUE TO PCR RIBOTYPE 027 IN EUROPE

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Recent outbreaks of *Clostridium difficile*-associated diarrhoea (CDAD) 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, Japan and Europe. Definitions have been proposed by the European Centre of Disease Prevention and Control (ECDC) to identify severe cases of CDAD and to differentiate community-acquired cases from nosocomial CDAD (http://www.ecdc.europa.eu/documents/pdf/Ci_dif_v2.pdf). CDAD is mainly known as a healthcare-associated disease, but it is also increasingly recognised as a community-associated disease. The emerging strain is referred to as North American pulsed-field type 1 (NAP1) and PCR ribotype 027. Since 2005, individual countries have developed surveillance studies to monitor the spread of this strain. *C. difficile* type 027 has caused outbreaks in England and Wales, Ireland, the Netherlands, Belgium, Luxembourg, and France, and has also been detected in Austria, Scotland, Switzerland, Poland and Denmark. Preliminary data indicated that type 027 was already present in historical isolates collected in Sweden between 1997 and 2001.

Introduction

A highly virulent variant of *Clostridium difficile* is emerging throughout Europe. This strain is characterised as toxinotype III, North American pulsed field gel electrophoresis type 1 (NAP1),

restriction endonuclease analysis group BI and PCR ribotype 027 [1,2,3]. The type 027 strain carries the binary toxin gene, has an 18bp deletion in the regulatory gene *tcdC*, and a 1bp deletion at position 117 of *tcdC*, resulting in a frameshift mutation that potentially allows for larger amounts of toxins to be produced. It is assumed that the increased virulence of this strain is associated with higher amounts of toxin production [4]. Clinical response rates are reduced following treatment with metronidazole or vancomycin [5,6]. This 027 type strain was first isolated in 1988 in France and is considered a 'historical isolate', since it was susceptible to fluoroquinolones and erythromycin [7]. It only accounted for sporadic cases of *C. difficile*-associated disease (CDAD) until 2002. It has been suggested that the recent acquisition of resistance to the newer fluoroquinolones by the 027 strain was the major reason for its wide dissemination [1,2] although this phenotype is not uncommon in other *C. difficile* strains [8]. Alternatively, increased virulence resulting in pronounced diarrhoeal symptoms may have promoted spread and cross-infection within healthcare institutions. Since 2002, it had caused major epidemics of CDAD in hospitals in Canada and also in the United States [1,2,3].

Background

CDAD occurs most often in people whose normal gut flora has been disturbed, for example during antibiotic treatment. The

clinical manifestation of CDAD can range from diarrhoea to severe pseudomembranous colitis, with a mortality of up to 30% [1]. CDAD is mainly known as a healthcare-associated disease, but it is increasingly recognised as a community-associated disease. At the 17th European Congress for Microbiology and Infectious Disease (31 March-3 April 2007) in Munich, results of a German pilot study were presented. It revealed a high CDAD incidence of 9.3% among 703 patients with diarrhoea visiting general practitioners in the period from August to December 2006 in Germany. *Salmonella enteritica* was cultured in 4.8% and *Campylobacter* in 3% of those patients [9].

The diagnostic methods routinely employed in different European laboratories today are not standardised and vary significantly [1,10]. Most laboratories prefer to detect *C. difficile* specific toxins in faeces. Faeces toxin detection can be performed either by cell cytotoxicity assay or immunological detection. The former is the gold standard, but requires up to two days. Various enzyme-immunoassays are available for immunological detection of *C. difficile* toxins, but their sensitivity and specificity varies enormously. Therefore, a study funded by the European Union (EU) has been launched in order to improve diagnostics of CDAD (LSHE-CT-2006-037870: European approach to combat outbreaks of CDAD by development of new diagnostic tests).

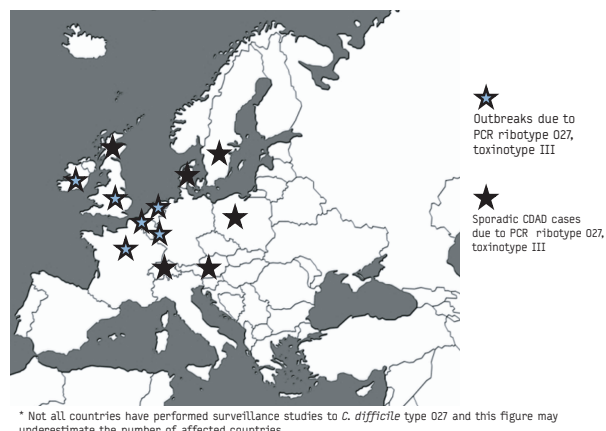
Surveillance efforts

The European Study Group for *Clostridium difficile* (ESGCD) performed a two-month surveillance study in 2005 on the prevalence of CDAD due to *C. difficile* O27 in 12 EU member states [11]. Based on these data and the recently published background document supported by the ECDC, individual countries have developed surveillance studies to the spread of type O27 in their country [1]. *C. difficile* type O27 causes outbreaks in the United Kingdom (UK) (since 2003 [12]), the Netherlands (since 2003 [13,14]), Belgium (since 2003 [15,16]), France (since 2006 [17,18]), and has also been detected in Austria (2006 [19]), Japan (2005 [20]) and Ireland [21]. In addition, it has been found in Switzerland (AF Widmer, R Frei, M Rupnik, personal communication), Luxembourg (P Reichert, E Kuijper, personal communication), Poland (H Pituch, E Kuijper, personal communication), and Denmark [22] (Figure). Preliminary data presented at the 2nd International *Clostridium difficile* symposium in Maribor, Slovenia (6-9 June, 2007) indicated that type O27 was present in three of more than 1,500 historical isolates collected in Sweden between 1997 and 2001. These strains were sensitive to fluoroquinolones and resemble the pre-outbreak type O27 strains in the United States [2] and France [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 (*UK hospitals are managed by acute trusts; for a detailed definition see: <http://www.info.doh.gov.uk/nhsfactsheets.nsf/vwHelp/Acute%20trusts?OpenDocument>*) since January 2004. Some 55,681 cases were reported in 2006. This represents an 8% increase in CDAD cases from 2005 to 2006, after a 17% increase from 2004 to 2005. The mandatory surveillance is operated by the Health Protection Agency (HPA) on behalf of the Department of Health (DH). 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 [23]. CDAD incidence rates of individual trusts are publicly reported each year by the DH [24]. Through its network of regional laboratories

FIGURE

Distribution of *C. difficile* ribotype O27 in Europe* as of June 2007



* Not all countries have performed surveillance studies to *C. difficile* type O27 and this figure may underestimate the number of affected countries

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. In an allocated week, local hospitals within each of the nine HPA regions were asked to submit a maximum of 10 *C. difficile* toxin-positive stools to their regional HPA laboratory to culture *C. difficile*. Isolates of putative *C. difficile* were then forwarded to the ARL for confirmation of identity, susceptibility testing against eight antimicrobial agents and typing by the PCR ribotyping method. The findings have recently been published in *Eurosurveillance* [25]. A laboratory surveillance network in England was established in 2007 to facilitate the early investigation of clusters of CDAD, particularly those associated with severe symptoms.

In October 2005, the National Institute for Public Health and the Environment (RIVM) in the Netherlands published specific CDAD ribotype O27 guidelines for infection control and treatment to be used by hospitals and nursing homes in response to the outbreaks in the Netherlands. 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 [26]. The results of the first year of surveillance are currently in press [27].

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. A national, prospective surveillance of CDAD incidence among hospitals will be implemented in 2007 and will include a sampling scheme in order to better assess the geographical dissemination of *C. difficile* strains.

One case of *C. difficile* O27 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 O27. Mandatory surveillance in line with the English system has been initiated in Scotland in 2006. Data on the incidence of *C. difficile* O27 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.

TABLE

C. difficile type O27 in 11 European countries (due to differences in surveillance the data cannot be directly compared).

Country	Survey period	Total number of inhabitants / hospitals / hospital beds	Number of hospitals positive for O27 / number of hospitals investigated for O27	Number of nursing homes positive for O27	Number of O27 strains / Total number of strains tested	Mortality attributable to CDAD	Updates available at:
England and Wales	2005-2006	53.4 million inhab/ England: 172 acute trust hosp/135,794 beds; Wales: 95 /11,500	94/170 (55.3%)	n.a	971/n.a	n.a	http://www.hpa.org.uk/infections/topics_az/clostridium_difficile/default.htm
Scotland	2006-2007	5.1 million/ 261 (incl. 45 acute hosp)/ 29,000	1/9 (11%)	0	1/103 (1%)	n.a	http://www.hps.scot.nhs.uk/haic/sshqip/clostridiumdifficile.aspx
Ireland	2006	4.2 million/61/10,000	7/7 (100%)	2	81/350 (23.1%)	n.a	http://www.ndsc.ie/hpsc/A-2/Gastroenteric/CDifficile
France	Jan2006 - April 2007	64 million/ca. 2,800/ca. 460,000	40/164 (24.3%)	4	277/471 (58.8%)	4% (Northern France only)	http://www.invs.sante.fr/raisin
The Netherlands	2005-2006	16.4 million/129/53,000	20/50 (40%)	7	218/863 (25.2%)	6.3%	http://www.rivm.nl/cib/infectieziekten/Clostridium_difficile/Clostridium_difficile_draaiboek.jsp
Belgium	2005-2006	10 million/113/51,640	38/78 (48.7%)	n.a	190/814 (23%)	n.a	http://www.belgianinfectioncontrolsociety.be
Poland	2005	38 million/781/184,000	1/1 (100%)	n.a	1/175 (0.6%)	na	
Austria	2006	8.2 million/ 264/ 63,248	1/20 (5%)	n.a	1/102 (1%)	n.a	
Luxembourg	2006	0.45 million/10/2100	4/10 (40%)	n.a	18/75 (24%)	n.a	
Switzerland	2005-2006	7.3 million/337/28,080	3/11 (27%)	1	4/231 (1.7%)	0%	
Denmark	Nov 2006-March 2007	5.4 million/ 69/22,604	n.a	n.a	6 (pilot study)	Study in progress	

n.a: data not available.

The available results from the surveillance efforts of 11 European countries are summarised in the Table. As methodology, time period and geographical coverage of surveillance differ significantly from one country to another, these results are qualitative and cannot be used for purposes of comparison. A new surveillance study among all European member states, planned for 2007-2008, is currently being developed by ECDC in collaboration with ESGCD, a study group for *Clostridium difficile* set up by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). In addition, a specific surveillance programme (CDAD-KISS) has recently been launched in Germany.

Conclusion

C. difficile type O27 has been detected in an increasing number of European countries. This could either be due to the fact that more countries have started surveillance surveys or an indication that type O27 is spreading rapidly. As yet, type O27 has affected healthcare facilities in 11 EU member states and in Switzerland (Figure). Increased awareness is necessary in all member states and surveillance studies should be performed with uniform definitions, as proposed by ECDC [1]. A guidance document for infection control measures has recently been prepared by international experts together with ECDC [28]. It is unknown how many CDAD cases in nursing homes and the community are due to type O27. The situation in those settings warrants more attention in the future.

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