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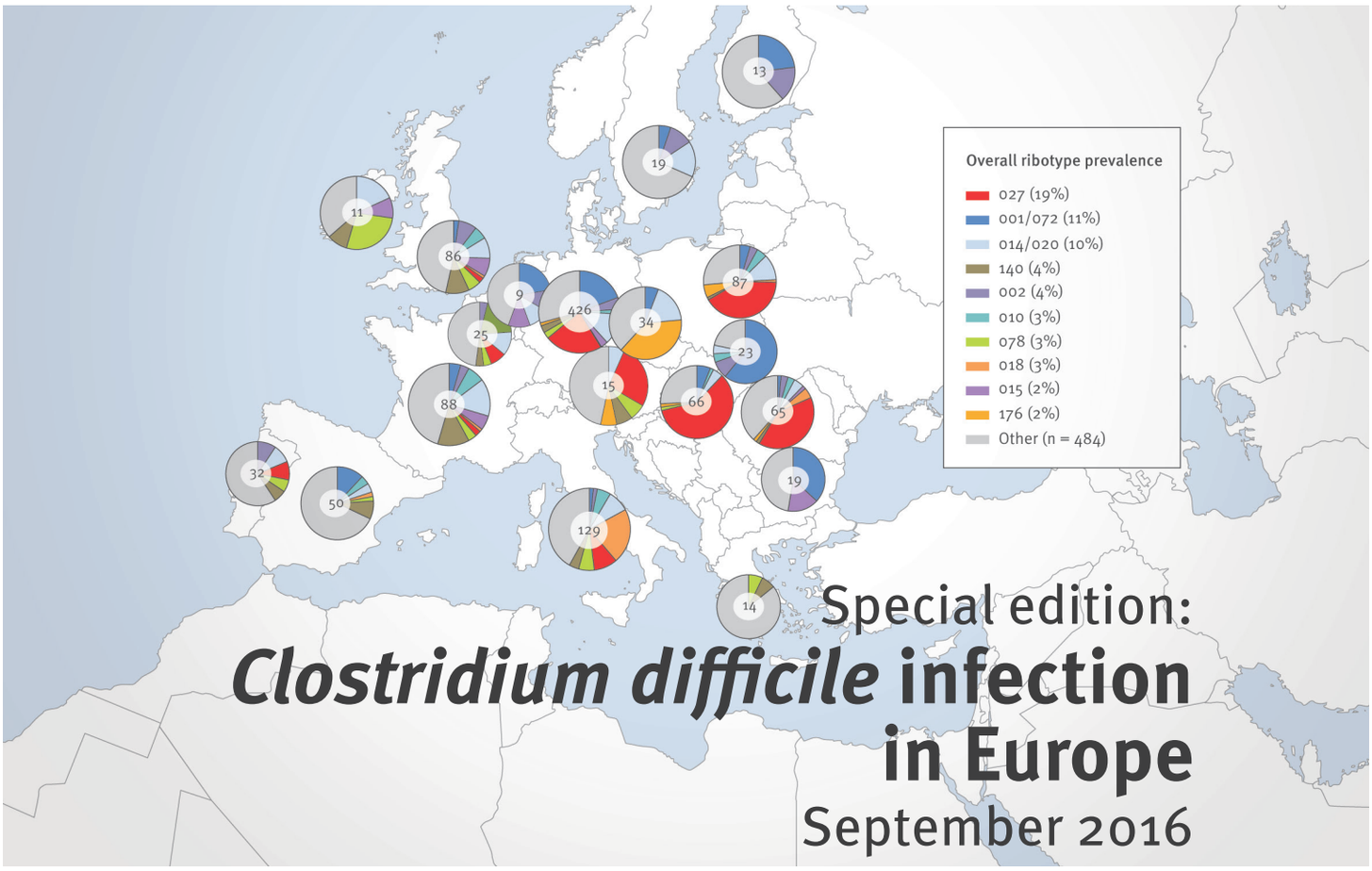

Special edition:

Clostridium difficile infection in Europe

September 2016

Featuring

- **Survey of diagnostic and typing capacity for *Clostridium difficile* infection in Europe, 2011 and 2014**
- **Standardised surveillance of *Clostridium difficile* infection in European acute care hospitals: a pilot study, 2013**
- **Enhanced surveillance of *Clostridium difficile* infection occurring outside hospital, England, 2011 to 2013**

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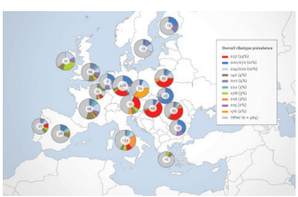
Contents

Editorial

Surveillance reports



Source: Davies KA, Ashwin H, Longshaw CM, Burns DA, Davis GL, Wilcox MH, on behalf of the EUCLID study group. Diversity of Clostridium difficile PCR ribotypes in Europe: results from the European, multicentre, prospective, biannual, point-prevalence study of Clostridium difficile infection in hospitalised patients with diarrhoea (EUCLID), 2012 and 2013. Euro Surveill. 2016;21(29):pii=30294. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.29.30294

Difficile indeed

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It has always lived up to its ominous species name: *Clostridium difficile*. It is difficult to culture, for example, and for this reason had for decades almost exclusively occupied hard-core devotees of anaerobic bacteria. In the 2000s, it also proved difficult to handle: outbreaks in hospitals began to be reported with increasing frequency in Europe and North America. Hypervirulent strains belonging to ribotype 027, and to a lesser extent 078, emerged that caused high morbidity and mortality among those infected, reviewed in [1,2]. Awareness rapidly increased and typing methods were fine-tuned. Investigations into risk factors for infection eventually led to identification of promising control measures, such as prudent antimicrobial drug stewardship, especially for those in risk groups.

The fact that the increase in frequency and severity of *C. difficile* infections was international led to discussions on the need to harmonise – and, if possible, standardise – methods and approaches for surveillance, diagnosis and strain typing. Two previous European reports provide the background to the work presented in the six papers forming this special issue of *Eurosurveillance*. These Europe-wide surveillance studies reported an increase in the mean incidence of *C. difficile* infections from 2.45 cases per 10,000 patient-days per hospital in 2005 to 4.1 in 2008 per 10,000 patient-days per hospital [3,4].

The special issue now at hand presents a mosaic of approaches, from an updated mapping of 'the European territory' to focused country-specific studies. What follows in this editorial is primarily a critical reading of the data, concentrating more on points that this author deems worthy of improvement or further attention.

Two European surveys look at available *C. difficile* infection (CDI) surveillance systems, and at laboratory capacity to diagnose CDI and type the responsible isolates [5,6]. While both identify several positive aspects, they also highlight room for improvement: the first, by Kola et al. based on data from 2011, shows that less than half of the responding 31 countries had a comprehensive, nationwide, ongoing CDI surveillance system, while in three of them, only severe cases were being notified [5]. The documented use of different definitions, including the distinction between healthcare- and community-associated (HA and CA) infections, poses an evident challenge for data comparison between countries. Perhaps even more critically, laboratory confirmation was included in 10 of the 18 analysed surveillance systems, and outcome only in five. Microbiological data, e.g. antimicrobial drug resistance phenotypes or molecular types, were regularly integrated with epidemiological data only in four countries, thus hampering immediate attempts to accurately identify potential outbreaks and dissemination routes. It will now be interesting to see to what extent these drawbacks will be overcome by the European Union (EU)/European Economic Area (EEA)-wide hospital-based CDI surveillance launched by the European Centre for Disease Prevention (ECDC): the surveillance protocol was published in 2015 [7], with data collection beginning in 2016.

The second survey, by van Dorp et al., compared European laboratory capacity to diagnose CDI and type the responsible isolates, in 2011 and in 2014, through the European *C. difficile* Infection Surveillance Network (ECDIS-Net) [6]. As already mentioned, laboratory capacity is crucial to detect and monitor the epidemiology of CDI and to detect the emergence of new strains. A total of 83 laboratories – that, unfortunately, could only be selected by convenience sampling– responded to the survey. The authors observed improvements in different aspects of the diagnostic approach between 2011 and 2014 in up to five laboratories per each improved aspect*. Comparison within a short span of three years may not have allowed a more promising extent of improvement. Nevertheless, some improvements could be seen when considering the use of diagnostic algorithms – classified as 'optimal', 'acceptable' or 'incomplete', although this classification was challenged by several participants. Here, a significant overall improvement, up to 31% or 46%, depending on bias assumptions, was observed. Identified barriers to improvements were, unsurprisingly, cost and lack of trained personnel. The existence of such barriers

in some European countries invites decisive European initiatives to overcome them. It should not be unreasonable to hope that European public health may also benefit from approaches (e.g. in funding) that have so clearly improved opportunities for European citizens in other areas of life, such as through strengthening Infrastructure for transport.

Harmonisation and standardisation of laboratory approaches and methods were another challenge. Building trust and capacity through persistent collaboration and proof-of-principle studies, e.g. in the form of ring trials that are as inclusive as possible, have been shown to help in similar contexts, as in [8].

Building on the two European surveys opening this issue [5,6], three CDI surveillance options were developed and piloted over a short period of three months in 37 acute care hospitals in 14 European countries: 'minimal' CDI surveillance (aggregated data); 'light' (including patient data for CDI); and 'enhanced' (with microbiological data for the first 10 CDI episodes for each hospital). In their paper, van Dorp et al. report a workload increasing, respectively, from 1.1 to 2.0 to 3.0 person-days per 10,000 hospital discharges, and that most responding hospitals found the light and enhanced options 'not difficult' [9].

Of the 14 European countries analysed, nine had already implemented CDI surveillance programmes, while five had not, and only two of the latter category declared that they would pursue it past the study's endpoint. The majority of hospitals were tertiary care hospitals: only five primary care hospitals participated. Unfortunately, only nine of the 14 participating countries took advantage of the offer for external quality assessment of strain typing, for reasons that are unclear. Of the 1,152 CDI episodes recorded by 'minimal' surveillance, only 23% included microbiological data in the 'enhanced' surveillance. This highlights once again laboratory data as a bottleneck towards a complete and high-value epidemiological picture necessary for timely control measures. The 'infamous' ribotype 027, though dominant (30% of all isolates), was identified in eight of the 14 participating countries, and with a widely varying frequency, ranging from 4% to 85% [9].

In the fourth European-wide report by Davies et al., a point-prevalence study that took place at two timepoints, in 2012 and 2013, investigators bypassed the problem of interlaboratory harmonisation and standardisation by relying on a single reference laboratory [10]. They ribotyped 1,196 isolates from 482 hospitals in 19 European countries and identified 125 different ribotypes. Ribotype 027 represented 19% of the total. In areas where 027 (or 176, but not other strains) was dominant, overall ribotype diversity was low. This finding illustrates the ability of these two epidemic strains to very successfully occupy their species' ecological niche. On the other hand, increased ribotype diversity was seen in a specific patient age-group: those over 80 years' old. While in 2008, the most prevalent European ribotype was 078, in the 2012–13 study, it had dropped to only 3% – an almost threefold decrease, counter-mirroring the over threefold increase of 027. Interestingly, no distinct associations of specific ribotypes with either colonisation or infection were seen.

One final point from this study merits clinical and epidemiological attention: over 7% of isolates from infected patients belonged to ribotypes known to be non-toxinogenic. The authors therefore hypothesise multistrain infections, as indeed previously shown by others. The study also showed that, in addition to the presence of 'pan-European' ribotypes, some ribotypes did exhibit country- or region-specificity, emphasising the importance of adequate knowledge of local epidemiology for taking appropriate measures. Two papers in this issue, originating from different countries, complete the picture.

Fawley et al. present results from enhanced surveillance, comparing CA- and HA-CDI in England, from 2011 to 2013 [11]. They found ribotype 027 and recent antibiotic treatment significantly higher in the HA group, while ribotypes 002, 020 and 056 and no recent antibiotic treatment were more frequent among CA isolates. In contrast to the European-wide study by Davies et al., which, however, did not differentiate between HA- and CA- CDI, ribotype diversity decreased with increasing age among HA- as opposed to CA-CDI isolates. Of course, as the authors acknowledge, these comparisons rest on the assumption that the majority of elderly patients living in care homes did not routinely receive healthcare and thus will have rightly been categorised as CA cases. Finally, in patient groups with recent hospital contact, ribotype diversity was reduced – as might be expected from exposure to a more outbreak-prone environment, where one or a few epidemic strains would predominate.

Data from enhanced surveillance in the Czech Republic in 2014 are presented by Krutova et al. [12]. Voluntary participation of 18 hospitals, covering 30% of the country's hospital bed capacity, yielded an incidence of 6.1 cases per 10,000 patient bed-days for both CA- and HA- CDI, and 774 isolates that were ribotyped. Among 33 known and 37 novel ribotypes observed, ribotypes 176 and 001 predominated (24% and 29%, respectively). Further subtyping among these two ribotypes, by the more discriminatory multilocus variable-number tandem-repeat analysis (MLVA), revealed clonal clusters of 176 and 001 that were common in 11 and seven hospitals, respectively. This could indicate patient-to-patient spread, though this was not specifically investigated. Frequent use of 'suboptimal' diagnostic algorithms, and low testing frequency when funds were limited, were identified weaknesses. However, an encouraging aspect of this report was the steadily increasing participation of Czech hospitals in such studies: from three in 2008, to 10 in 2012–13 and 18 in in 2014. Such increasing engagement confirms

that time, persistence, positive experience, as well as intracountry concerted efforts, can lead to a positive outcome.

Taken together, the studies presented in this issue, in concert with those that had prepared the ground for them and those that doubtless will follow, are praiseworthy as they contribute to both a raised awareness and a more solid documentation of a field fraught with difficulties. From the perspective of a benefit to public health, however, it will be useful to see to what degree such extensive, harmonised and/or standardised surveillance and typing will lead to better control of CDI and further reduction of outbreaks as well as sporadic cases.

* Author's correction

The sentence was modified on 26 July 2016 at the request of the author, to reflect more accurately the data the sentence summarises.

Conflict of interest

None declared.

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Survey of *Clostridium difficile* infection surveillance systems in Europe, 2011

A Kola [1], C Wiuff [2], T Akerlund [3], BH van Benthem [4], B Coignard [5], O Lyytikäinen [6], D Weitzel-Kage [1], C Suetens [7], MH Wilcox [8 9], EJ Kuijper [10], P Gastmeier [1], on behalf of members of ECDIS-Net [11]

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To develop a European surveillance protocol for *Clostridium difficile* infection (CDI), existing national CDI surveillance systems were assessed in 2011. A web-based electronic form was provided for all national coordinators of the European CDI Surveillance Network (ECDIS-Net). Of 35 national coordinators approached, 33 from 31 European countries replied. Surveillance of CDI was in place in 14 of the 31 countries, comprising 18 different nationwide systems. Three of 14 countries with CDI surveillance used public health notification of cases as the route of reporting, and in another three, reporting was limited to public health notification of cases of severe CDI. The CDI definitions published by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the European Centre for Disease Prevention and Control (ECDC) were widely used, but there were differing definitions to distinguish between community- and healthcare-associated cases. All CDI surveillance systems except one reported annual national CDI rates (calculated as number of cases per patient-days). Only four surveillance systems regularly integrated microbiological data (typing and susceptibility testing results). Surveillance methods varied considerably between countries, which emphasises the need for a harmonised European protocol to allow consistent monitoring of the CDI epidemiology at European level. The results of this survey were used to develop a harmonised EU-wide hospital-based CDI surveillance protocol.

Introduction

Since 2000, a considerable increase in the number of *Clostridium difficile* infections (CDIs) leading to substantial morbidity, mortality and attributable costs has been observed, at least in North America and Europe [1]. Changes in the epidemiology of CDI have been mainly attributed to the emergence of a new hypervirulent strain called PCR ribotype 027, causing numerous outbreaks in North America and Europe [2,3] and, to a lesser extent, PCR ribotype 078 [1,4,5]. In addition, patients not previously considered to be at risk for the disease (e.g., without recent antibiotic therapy or hospitalisation) have also been described [1,6-8]. The European CDI study (ECDIS), initiated and funded by the European Centre for Disease Prevention and Control (ECDC), showed that the incidence of CDI varied from hospital to hospital [9]. In 2008, a weighted mean incidence of 4.1 cases (range: 0.0–36.3) per 10,000 patient-days per hospital reported by the ECDIS study was almost 70% higher than that reported in a previous European surveillance study in 2005 (2.45 cases per 10,000 patient-days per hospital, range: 0.13–7.1) [9,10]. ECDIS also revealed the contribution of strains other than PCR ribotype 027 and that some of these strains, notably PCR ribotypes 015, 018 and 056, could cause severe CDI.

In response to the emerging problems associated with *C. difficile*, an ECDC working group published background information about the changing epidemiology of CDI, CDI case definitions and surveillance recommendations [2]. To support European Union (EU)/European

Table

Characteristics of European *Clostridium difficile* infection surveillance systems, 2011 (18 surveillance systems from 14 countries)

Country	Name	Participants	General remarks	Epidemiological data	Microbiological data
Austria	No name	All H/L/G	M C+P Lb+Cb only sevCDI	Total number CDI-days	RTcp AST
Belgium	National Surveillance of Infections in Hospitals (NSIH)	110 H	M P Cb Periodic (6 months a year)	HA-CDI: I (1,000 pa/6 months) Id (10,000 pd/6 months) Severe CDI: ICU-adm/death within 30 days related to CDI	TcdT (fp/sevCDI /ob) AST (fp/sevCDI /ob)
Bulgaria	BGCDISS	6 H/3 L	V C+P Lb+Cb	HA-CDI: I (10,000 pa) Id (10,000 pd)	RTcp No AST
Denmark	Surveillance of epidemic hypervirulent CD in Denmark	13 H/13 L	V C+P Lb	I (number of episodes/region)	RTag (sevCDI+MoxR)/ob) AST
Finland-1	National Infectious Diseases Register	All L	M C+P Lb	I (100,000 inh)	RTag (sevCDI/ob) No AST
Finland-2	Finnish Hospital Infection Programme (SIRO)	12 H	V C+P Cb	HA-CDI: I (100 pa) Id (1000 pd) Severe CDI: ICU-adm/surgery/ death within 30 days related to CDI	None
Finland-3	National Hospital Discharge Register (HILMO)	57 H	M C (retrosp.) ICD 10-based	I (CDI hospitalisations/ 100,000 inh)	None
France	Healthcare acquired Infections Early warning and Response system	100 H/115 L/ 10 N	M C+P Cb only sevCDI/ob	Severe CDI: Total number I (1,000 pa) Id (10,000 pd)	RTag (sevCDI/ob) AST (sevCDI/ob)
Germany-1	CDAD-KISS	126 H	V C+P Cb	HA-CDI/severe CDI: I (100 adm) Id (1,000 pd)	None
Germany-2	SurvNet	About 2000 H	M C+P Cb only sevCDI/ ribotype 027	Severe CDI: Total number I (100,000 inh/ ICU-adm/surgery/ death within 30 days related to CDI)	RTcp (sevCDI/ob) No AST
Hungary	Epidemiological Control System and Information System (EFRIR)	35 H / 14 L	M C+P Lb+Cb	Total number	RTag (sevCDI/ob) No AST
Ireland-1	Notifiable *C. difficile* Surveillance	48 H/37 L / all G from 8 public health regions	M C+P Cb	I (100,000 inh)	None
Ireland-2	*C. difficile* Enhanced Surveillance	34 H/34 L	V C+P Cb	HA-CDI: Id (10,000 pd) Severe CDI: ICU-adm/ surgery related to CDI	None
The Netherlands	Sentinel surveillance of *C. difficile*	19 H/19 L	V C+P Lb+Cb	HA-CDI: I (CDI cases/pa) Id (CDI cases/pd)	RTag (fp) No AST
Sweden	National Laboratory-based CD Surveillance System	20 L	V C+P Lb	Total number	RTac (fp/sevCDI /ob) AST (fp)
UK-England	HCAI Data Capture System	167 NHS Acute Trusts with 1–2 H each	M C+P Lb	All types of CDI: Id (adm>65 y/1,000 pd) HA-CDI: Id (cases>2 y/10,000 pd) Severe CDI: Death within 30 days related to CDI	RTca AST (fp)
UK-Northern Ireland	Enhanced HCAI Web-based Surveillance System	28 H/ 5 L/ 358 GP / 240 N / 237 R	M C+P Lb	HA-CDI and CA-CDI: Total number Id (1,000 pd)	RTcp/no AST
UK-Scotland	Scottish Mandatory Surveillance Programme for CDI	23 L and 14 NHS health boards including H/N/G	M C+P Lb+Cb	HA-CDI: Id (cases≥15 y/1000 pd)	RTag (fp/sevCDI/ob) AST (fp/sevCDI/ob)

ac: acrylamide; adm: admissions; ag: agarose; AST: antimicrobial susceptibility testing; C: continuous; CA: community associated; cp: capillary; Cb: case-based; CD; *Clostridium difficile*; CDI: *Clostridium difficile* infection; fp: fixed proportion; G: general practioners; H: hospitals; HA: healthcare associated; I: incidence; ICD-10: International Statistical Classification of Diseases 10th revision; ICU: intensive-care unit; Id: incidence density; inh: inhabitants; L: laboratories; Lb: laboratory-based; M: mandatory; MoxR: moxifloxacin resistance; N: nursing homes; ob: outbreaks; pa: patient admissions; pd: patient-days; R: residential homes; retrosp.: retrospective; RT: ribotyping; sevCDI: severe CDI; TcdC: typing of the *tcdC* gene; UK: United Kingdom; V: voluntary; y: years.

[a] Iceland and UK-Wales did not reply to the web-based questionnaire.

[b] Some countries had more than one surveillance system in parallel. Where relevant, they are shown with the suffixes -1, -2 and -3.

Economic Area (EEA) Member States in increasing their capacity for CDI surveillance, ECDC also initiated and funded a new project – ECDIS-Net – to develop a European surveillance protocol and enhance laboratory capacity for diagnosis and typing of *C. difficile* in EU/EEA Member States.

In 2011, a survey of existing CDI surveillance systems in European countries was performed as part of the ECDIS-Net project. The results of this survey, presented here, were later used to develop a standardised pan-European CDI surveillance protocol, which was tested in a three-month pilot study in 2013 [11]. Data collection in the ECDC-coordinated Europe-wide hospital-based CDI surveillance, using a finalised version of this piloted protocol, began on 1 January 2016 [12].

Methods

National coordinators for this study were identified through the members of ECDC's Healthcare-Associated Infections surveillance Network (HAI-Net) and via representatives for the ECDIS study [9]. A link to a web-based questionnaire was sent to these national coordinators to assess the characteristics of existing CDI surveillance systems in European countries. If the national coordinators indicated that CDI was under surveillance in their country, the surveillance protocols were requested and used to augment the information obtained via the questionnaire. Information on the national CDI surveillance systems was entered using a web-based electronic form designed for the purpose of this study.

Results

Between 6 June and 15 July 2011, 33 of the 35 national coordinators approached from 31 European countries responded to the web-based questionnaire (Iceland and Wales did not respond). Four surveillance systems were excluded from further analysis, as they were not ongoing, comprehensive nationwide surveillance systems, i.e. they were completed one-off studies (two studies from Spain), only regional (Switzerland) or focused only on outbreaks (one system of the Netherlands). In 14 countries, the national coordinators indicated that surveillance of CDI was in place. Of these, surveillance protocols were available from 10 surveillance systems. Thus, 18 CDI surveillance systems from 14 European countries (Austria, Belgium, Bulgaria, Denmark, Finland, France, Germany, Hungary, Ireland, the Netherlands, Sweden and three countries of the United Kingdom (UK), England, Northern Ireland and Scotland) remained available for analysis. Of the 18 surveillance systems, all but one reported national CDI rates annually.

General characteristics of *C. difficile* infection surveillance systems

An overview of the European CDI surveillance systems is given in the Table. In summary, 11/18 surveillance systems used mandatory reporting and seven used voluntary reporting of cases. The majority (16/18) of the surveillance systems were continuous and prospective, one was periodical and prospective (Belgium), and one was retrospective (Finland-3). In three countries (Germany, Ireland, the Netherlands), two surveillance systems were run in parallel, (shown with the suffixes -1 and -2). In Finland, there were three parallel systems (Finland-1, -2 and -3). Parallel systems were also in place in the three parts of the United Kingdom that took part in the survey (England, Northern Ireland and Scotland). In Finland, Germany and Ireland, one surveillance system was limited to (legally required) public health notification of CDI cases, whereas additional systems collected laboratory-based data and enhanced epidemiological data on a voluntary basis. Public health notification of CDI was also carried out in Austria, Denmark and Hungary.

In Austria, France and Germany-2, surveillance of CDI targeted severe cases only. All surveillance systems included CDI in hospitalised patients, but 10/18 systems also included patients with community-acquired CDI. CDI case ascertainment was case-based (including clinical evaluation) in 7/18 systems, laboratory-based (relying on positive test results for toxin-producing *C. difficile*) in 5/18 systems or a combination of both in an additional 5/18 surveillance systems. Only Finland-3 used the International Statistical Classification of Diseases 10th revision (ICD-10)-based discharge coding [13] to find cases of CDI.

Definitions of *C. difficile* infection

The definitions used for CDI surveillance are summarised in the Box.

The majority (12/18) of the surveillance systems used the ECDC and CDC case definition of CDI [2,14], 4/18 used other definitions and 2/18 did not use a specific case definition (but relied instead on the diagnosis of the attending physician and a positive laboratory test result for toxigenic *C. difficile*). More detailed definitions for community-associated CDI, community-onset of healthcare-associated CDI and healthcare-onset of healthcare-associated CDI were used by 9/18 (ECDC definition: 7/9, other definitions: 2/9). Definitions differing from ECDC's for community-associated CDI, community-onset healthcare-associated CDI and healthcare-onset healthcare-associated CDI used a time point of ≥ 72 hours or › 3 days (i.e. on or after day 4 of admission) instead of ≥ 48 hours between admission and onset of symptoms to distinguish between community- and healthcare-associated CDI.

In 13/18 surveillance systems, there was a definition for severe cases of CDI (ECDC definition: 5/13, other definitions: 8/13) and in 11/18 systems, there was also a definition for recurrence of CDI (ECDC definition: 9/11, other definitions: 2/11). Definitions differing from ECDC's definition for severe/complicated course of CDI used additional criteria such as bloody diarrhoea, temperature › 38.5 °C, white cell count › 15 × 109/L, decreased kidney function or hypo-albuminaemia (‹ 30

Box

Definitions, including surveillance system-specific definitions[a], for surveillance of *Clostridium difficile* infections

CDI case A patient to whom one or more of the following criteria applies: 1. diarrhoeal stools or toxic megacolon AND a positive laboratory assay for C. difficile TcdA and /or TcdB in stools or a toxin-producing C. difficile organism detected in stool via culture or other means; 2. pseudomembranous colitis revealed by lower gastrointestinal endoscopy; 3. colonic histopathology characteristic of CDI (with or without diarrhoea) on a specimen obtained during endoscopy, colectomy or autopsy.
Differing definitions: Finland-1: Detection of C. difficile organism/DNA/RNA/toxin in a clinical sample. Finland-3: International Classification of Diseases (ICD)-10 codes A04.7 and K52.8 specific for Clostridium difficile-associated disease. UK-England: Diagnoses on the basis of tests for C. difficile toxins A and B on diarrhoeal stool samples. Positive results on the same patient within 28 days of the first specimen are regarded as a single episode. All cases are reported regardless of location of the patient at the time the specimen was taken, i.e. regardless of whether the patient was in a hospital or another setting. Diarrhoeal stools are defined as 'those that take the shape of their container'. One (unexplained) diarrhoeal episode is sufficient to qualify for a diagnosis of CDI if the laboratory test is supportive. UK-Northern Ireland: A patient aged two years and over from whom a diarrhoeal specimen is tested positive for C. difficile.
Community-associated CDI Onset of CDI outside a healthcare facility (HCF) or within 48 hours following admission to a healthcare facility without residence in/ discharge from a healthcare facility within the previous 12 weeks.
Differing definitions: Finland-2 and Germany-1: Onset of CDI in an outpatient or inpatient within 72 hours after admission to the facility.
Community-onset of healthcare-associated CDI Onset of CDI in the community within 4 weeks following discharge from a healthcare facility.
Healthcare-onset of healthcare-associated CDI Onset of CDI at least 48 hours (›48 hours) following admission to a healthcare facility
Complicated course of CDI (severe CDI case) A patient to whom any of the following criteria applies: 1. admission to a healthcare facility for treatment of community-associated CDI; 2. admission to an intensive-care unit for treatment of CDI or its complication (e.g. for shock requiring vasopressor therapy); 3. surgery (colectomy) for toxic megacolon, perforation or refractory colitis; 4. death within 30 days after diagnosis, if CDI is either the primary or a contributive cause.
Differing definitions: Austria: CDI requiring admission to an intensive-care unit/CDI requiring surgery/fatal cases of CDI. Germany: Instead of 1: Readmission because of recurrent CDI (points 2–4 as above) France: In addition: white cell count›20 × 103/mm3. Hungary: Death linked to CDI (based on death register). Ireland-2: 1. Admission to an intensive care unit for treatment of CDI or its complication (e.g. for shock requiring vasopressor therapy) and/ or 2. surgery (colectomy) for toxic megacolon, perforation or refractory colitis. The Netherlands: 1. Bloody diarrhoea and/or 2. pseudomembranous colitis and/or 3. diarrhoea in combination with dehydration and/or hypo-albuminaemia (‹30 g / L) 4. temperature›38 °C and white cell count›15 × 109/L. UK-England: Temperature›38.5 °C, white cell count›15 × 109/L, decreased kidney function, or evidence of colitis. UK-Scotland: In addition: Endoscopic diagnosis of pseudomembranous colitis (with or without toxin confirmation) persisting CDI where the patient has remained symptomatic and toxin positive despite two courses of appropriate therapy.
Recurrent CDI An episode of CDI that occurs ›2 weeks and≤ 8 weeks following the onset of a previous episode.
Differing definitions: UK-England: A positive specimen taken more than 28 days after the initial specimen is considered a new CDI episode. UK-Scotland: A new episode is defined as one occurring more than 28 days after the previous onset.

CDI: *Clostridium difficile* infection; UK: United Kingdom.
[a] Some countries had more than one surveillance system in parallel.
Source: [2,14]. Surveillance system-specific definitions: this study.

g/L). Definitions differing from those used by ECDC for recurrent CDI used a time lapse of between two and four weeks after the previous onset to distinguish between different episodes of CDI.

Collection of *C. difficile* infection surveillance data

In 5/18 surveillance systems, data collection was done only by laboratories, in 7/18 only by infection control teams, and in 5/18 by both. One surveillance system used hospital administration data only (Finland-3). In 8/18 surveillance systems, case-based data were collected by healthcare personnel (in 7/8 in combination with the infection control teams). In addition, general practitioners were engaged in surveillance data collection in Austria and UK-Scotland, as were public health doctors in Ireland-1. Only 3/18 surveillance systems relied solely on laboratory tests positive for CDI without additional patient data (Denmark, Finland-1, Sweden).

The collected data were pooled nationwide in 11/18 surveillance systems (Belgium, Bulgaria Finland-1, Finland-3, France, Hungary, Ireland-1, Ireland-2,

Sweden, UK-Northern Ireland and UK-Scotland), per district or health board in 9/18 systems (Austria, Denmark, Finland-1, Finland-3, France, Germany-2, Ireland-1, UK-Northern Ireland and UK-Scotland), per healthcare facility in 9/18 systems (Belgium, Bulgaria, Finland-2, France, Germany-1, Ireland-2, the Netherlands, UK-England, UK-Northern Ireland) and per unit within a healthcare facility in 2/18 systems (Finland-2, UK-Northern Ireland). In Finland-3 and Sweden, the collected data were also pooled per laboratory. Data about the size or type of the reporting healthcare facility were collected in 12/18 CDI surveillance systems, but not in the remaining six systems (Austria, Denmark, Finland-1, Germany-2, Hungary, Sweden). In 8/18 surveillance systems, even the speciality of the reporting unit or department was known. Most of the surveillance systems collected patient data: age and sex of CDI cases were reported in 16/18 surveillance systems, the date of onset of CDI in 13/18 systems and the date of admission in 11/18 systems. Only one surveillance system did not collect any patient data (Germany-1). Data about the history of CDI cases were collected in 6/18 surveillance systems (number of previous hospital admissions: 2/6, number of previous episodes of CDI: 4/6; recurrent CDI: 5/6) and data about the outcome of CDI (death within 30 days) were collected in 5/18 systems.

Reporting of *C. difficile* infection surveillance data

CDI surveillance results were periodically reported in 16/18 surveillance systems (ranging from daily reports in UK-Northern Ireland to annual reports in 9/18 systems); only 2/18 surveillance systems did not report the results at regular intervals (Finland-3, Germany-2). All 18 surveillance systems published their reports nationally, but in 6/18 and 3/18 surveillance systems, there were additional regional and local reports, respectively. Most (12/18) of these reports were available to the public and healthcare professionals; only 4/18 and 2/18 surveillance systems published reports that solely targeted healthcare professionals or the public, respectively. Surveillance results were stratified in 8/18 surveillance systems, mostly by geographical region (4/8) or type of healthcare facility (4/8). More details, including denominators and calculated CDI rates, are given in the Table.

Typing

Typing of *C. difficile* was performed by national reference laboratories in 13 European countries with CDI surveillance, PCR ribotyping (either agarose: 8/13, acrylamide: 1/13 or capillary gel-based: 4/13) being the preferred method. Only one reference laboratory also used *tcdC* typing (Belgium). For the purposes of surveillance, typing was done in 13/18 European surveillance systems with varying criteria for submitting strains for further typing: severe CDI (9/13), outbreaks (7/13), isolates resistant to moxifloxacin (Denmark) or a more systematic sampling design selecting (4/13), e.g. the first five strains of each semester, i.e. each half of the year (Belgium), all strains of selected calendar periods (Sweden, UK-Scotland) or selected hospitals (the Netherlands). An overview is given in the Table. A more detailed analysis was performed by another ECDIS-Net survey in 2011 and 2014 of diagnostic and typing capacity for CDI in Europe: the results of which are also reported in this issue [15].

Susceptibility testing

There were no official recommendations for routine susceptibility testing of *C. difficile* isolates in any of the European countries taking part in ECDIS-Net, but susceptibility testing results were included in 7/18 CDI surveillance systems analysed. Conditions leading to susceptibility testing were the surveillance of antimicrobial resistance itself (5/7), severe CDI cases (4/7) or outbreaks of CDI (3/7).

Discussion

This survey showed that 14 of 31 European countries surveyed conducted some kind of CDI surveillance in 2011. The majority of the 18 existing European nationwide CDI surveillance systems were continuous and prospective, and captured CDI cases by standardised case definitions targeting the clinical symptoms of CDI and/or laboratory diagnosis of CDI, and all of them included CDI in hospitalised patients. However, there were interesting differences between these systems. In 11/18 of European countries with CDI surveillance, surveillance was mandatory, either by mandatory reporting of laboratory and/or clinically confirmed cases or by public health notification of CDI. Whether surveillance should be based on mandatory or voluntary reporting of confirmed cases is still under discussion [16-18]. Opponents of mandatory reporting argue that especially in combination with public reporting of surveillance results and financial penalties, it may lead to systematic under-reporting of cases.

An important issue for surveillance purposes is the definition of CDI cases. These definitions should be valid, specific, easily understood, generally applicable and meet the requirements of different clinical settings, ideally across borders. Moreover, they should allow the comparison of local, regional, national and international infection rates [19]. The definitions proposed by the study group for *C. difficile* of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and ECDC [2] are in agreement with those of the United States Centers for Disease Control and Prevention (CDC) [2,14]. Most of the European CDI surveillance systems adhere to these definitions, but difficulties are encountered in differentiating between community- and healthcare-associated cases of CDI. Some surveillance systems do not make any distinction between the two types of cases (for instance, when only laboratory data are used), while others use different time points for differentiating between the two. Stratification of community-associated and healthcare-associated CDI cases may permit recognition of changes in epidemiology, e.g. an increase in the

number of community-associated cases of CDI possibly caused by 'hypervirulent' *C. difficile* strains [1,20,21]. For feasibility reasons, the definitions of community- and healthcare-associated cases of CDI could be simplified, e.g. by adjusting the threshold time between the two types of cases to three days or later instead of 48 hours. However, regardless of the threshold used, variable proportions of CDI cases defined as community-associated CDI cases may in fact be linked to recent hospitalisation.

In order to meet the ECDC CDI case definitions, most surveillance systems used laboratory reporting and identification of CDI cases by attending healthcare personnel and/or infection control practitioners; few relied solely on laboratory test results. Only one of the Finnish surveillance systems used ICD-10 coding of CDI supplied by hospital administrations. In comparison with surveillance using CDI case definitions, surveillance using ICD coding has shown to be less sensitive [22,23]. In Finland, three different surveillance systems for CDI are run in parallel and so may compensate for their respective limitations.

All surveillance systems reporting hospital-associated CDI cases express CDI rates as incidence rate (per number of patient admissions within a given surveillance period) or incidence density (per number of patient-days). However, different orders of magnitude are used (100 or 1,000 admissions and 1,000 or 10,000 patient-days). Apart from that, surveillance systems only reporting the total number (i.e. community-associated and hospital-associated combined) of CDI cases mostly calculate the incidence per number of inhabitants; only a few exceptions just give the cumulative number of CDI cases. According to published recommendations and for better comparison, the incidence density of healthcare-associated and community-associated CDI should be expressed per 10,000 patient-days and 100,000 inhabitants, respectively [14,19].

More than half of the European CDI surveillance systems presented their findings pooled, i.e. without any further stratification. Unfortunately, only a few surveillance systems provided sender-specific analyses. This would, however, be very important to inform interventions at local level and may help to reduce infection rates [24].

Microbiological data may be an important supplement to epidemiological surveillance data and allow deeper insights into epidemiological changes. In our survey, however, strain typing and susceptibility testing were mainly restricted to outbreaks of CDI or severe cases of CDI; only a few surveillance protocols included typing or susceptibility testing on a regular basis. Although lacking the discriminatory power to study outbreaks, PCR ribotyping is the most adopted *C. difficile* typing methodology in European reference laboratories. International standardisation of ribotyping methods would allow comparability and reproducibility between countries. Capillary-based ribotyping offers the opportunity to achieve these aims, as results are easier to interpret and to exchange than those of conventional agarose-based ribotyping [25-27].

The main limitations of microbiological testing for *C. difficile* are financial, and shipment of strains to reference laboratories for typing may be hampered by the fact that many laboratories perform toxin testing alone and do not culture *C. difficile*.

Published recommendations of ECDC and the United States Centers for Disease Control and Prevention (CDC) are that CDI surveillance should be conducted for at least all inpatients to monitor healthcare-associated CDI, and healthcare-associated CDI rates should be expressed as number of cases per 10,000 patient-days [2,14]. A standardised European CDI surveillance protocol should be used to allow meaningful intercountry comparisons of CDI incidence rates and for follow-up of the epidemiology of CDI at European level. Special emphasis should be given to the harmonisation of definitions of community-associated and healthcare-associated CDI, inclusion criteria for patients and CDI cases, criteria for typing *C. difficile* strains, denominator data, epidemiological case-based data and case-finding methods. In order to integrate microbiological test results into CDI surveillance, more frequent culture of *C. difficile* is required, and typing methods should be standardised. Harmonised systematic surveillance at national and European level is more likely to facilitate the identification of epidemiological changes and the optimal control of CDI. As a result of this survey, ECDC published a harmonised EU/EEA-wide hospital-based CDI surveillance protocol in May 2015 [12].

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Conflict of interest

Mark H Wilcox: received research work fees, and/or consulting fees and/or lecture fees from Actelion, Cubist, Astellas, Merck, Optimer, Sanofi-Pasteur, Summit, Astra-Zeneca, Cerexa, Nabriva, Novacta, Novartis, Pfizer, Corporation Roche, The Medicines Company, VH Squared, Abbott, bioMerieux, Da Volterra, European Tissue Symposium, Basilea, and Alere (paid to the department), and a clinical trial consultancy from Durata.

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All other authors: none declared.

Authors' contributions

The survey was designed by AK, DWK and PG, with support of BHB, BC, OL, JS and CW. EJK and MHW were the principle coordinators of ECDIS-Net, using support of CS from ECDC. AK and DWK performed data collection and data analysis, PG supervised data collection and data analysis. AK wrote the manuscript together with CW. All co-authors reviewed the manuscript.

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Survey of diagnostic and typing capacity for *Clostridium difficile* infection in Europe, 2011 and 2014

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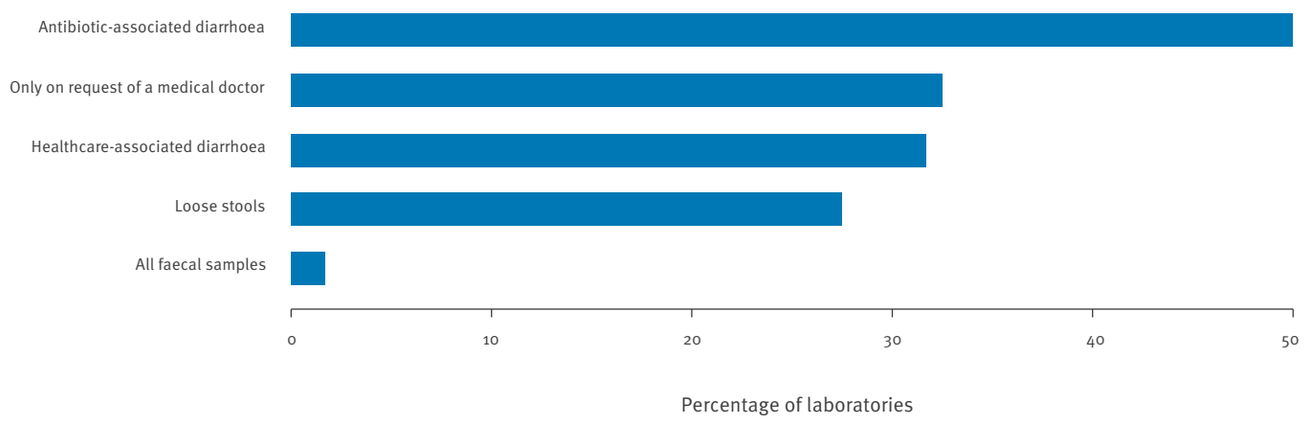
Suboptimal laboratory diagnostics for *Clostridium difficile* infection (CDI) impedes its surveillance and control across Europe. We evaluated changes in local laboratory CDI diagnostics and changes in national diagnostic and typing capacity for CDI during the European *C. difficile* Infection Surveillance Network (ECDIS-Net) project, through cross-sectional surveys in 33 European countries in 2011 and 2014. In 2011, 126 (61%) of a convenience sample of 206 laboratories in 31 countries completed a survey on local diagnostics. In 2014, 84 (67%) of these 126 laboratories in 26 countries completed a follow-up survey. Among laboratories that participated in both surveys, use of CDI diagnostics deemed 'optimal' or 'acceptable' increased from 19% to 46% and from 10% to 15%, respectively ($p < 0.001$). The survey of national capacity was completed by national coordinators of 31 and 32 countries in 2011 and 2014, respectively. Capacity for any *C. difficile* typing method increased from 22/31 countries in 2011 to 26/32 countries in 2014; for PCR ribotyping from 20/31 countries to 23/32 countries, and specifically for capillary PCR ribotyping from 7/31 countries to 16/32 countries. While our study indicates improved diagnostic capability and national capacity for capillary PCR ribotyping across European laboratories between 2011 and 2014, increased use of 'optimal' diagnostics should be promoted.

Introduction

Since 2003, Europe has been affected by outbreaks of *Clostridium difficile* infection (CDI) associated with the emergence of PCR ribotype 027/NAP1 [1]. A decade later, *C. difficile* was the microorganism responsible for 48% of healthcare-associated gastrointestinal infections in acute care hospitals across Europe [2]. Despite being frequent, CDI remains underestimated in most European countries [3]. Underdiagnosis mainly results from a lack of awareness among medical doctors of when to suspect that patients may have CDI and use of suboptimal diagnostic algorithms at local microbiological laboratories [3-5]. Reference tests, i.e. toxigenic culture and cell culture cytotoxicity assay (CCA), are not suitable for routine application due to their complexity and long turnaround time [6,7]. Rapid enzyme immunoassays (EIAs) to detect *C. difficile* toxins in faeces lack sensitivity [6,8]. Highly sensitive tests such as EIA detecting glutamate dehydrogenase (GDH) – a *C. difficile*-specific enzyme [9] – or nucleic acid amplification tests (NAATs) have insufficient specificity [6,10]. To overcome underdiagnosis and suboptimal performance of stand-alone tests, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) has recommended since 2009 testing loose stools using two-step algorithms that have a highly sensitive test as the first screening step and a highly specific test as the second confirmatory test [6,11]. The 'Bristol stool scores' [12] are commonly used to categorise stool consistencies and can be used to select samples for

Figure 1

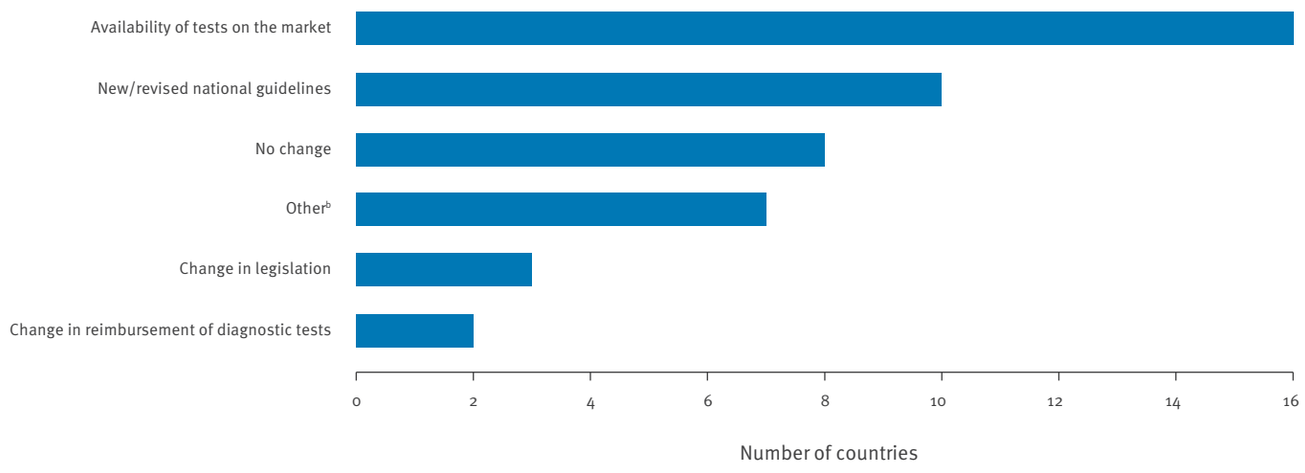
Criteria for selection of faecal samples tested for *Clostridium difficile* among responding local laboratories that participated in the European *Clostridium difficile* Infection Surveillance Network (ECDIS-Net) project in 2011 (n = 120)[a]



[a] Laboratories in 31 countries responded to the 2011 survey: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lichtenstein, Lithuania, Luxembourg, the Netherlands, Norway, Poland, Portugal, Romania, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom (not including Wales). Serbia did not participate in the European Clostridium difficile Infection Surveillance Network (ECDIS-Net) project in 2011. No laboratories in Slovakia and Wales were invited to participate by ECDIS-Net national coordinators in 2011.

Figure 2

Reported changes affecting national/subnational laboratory diagnostic capacity for *Clostridium difficile* infection between 2011 and 2014 in participating European countries (n = 32)[a]

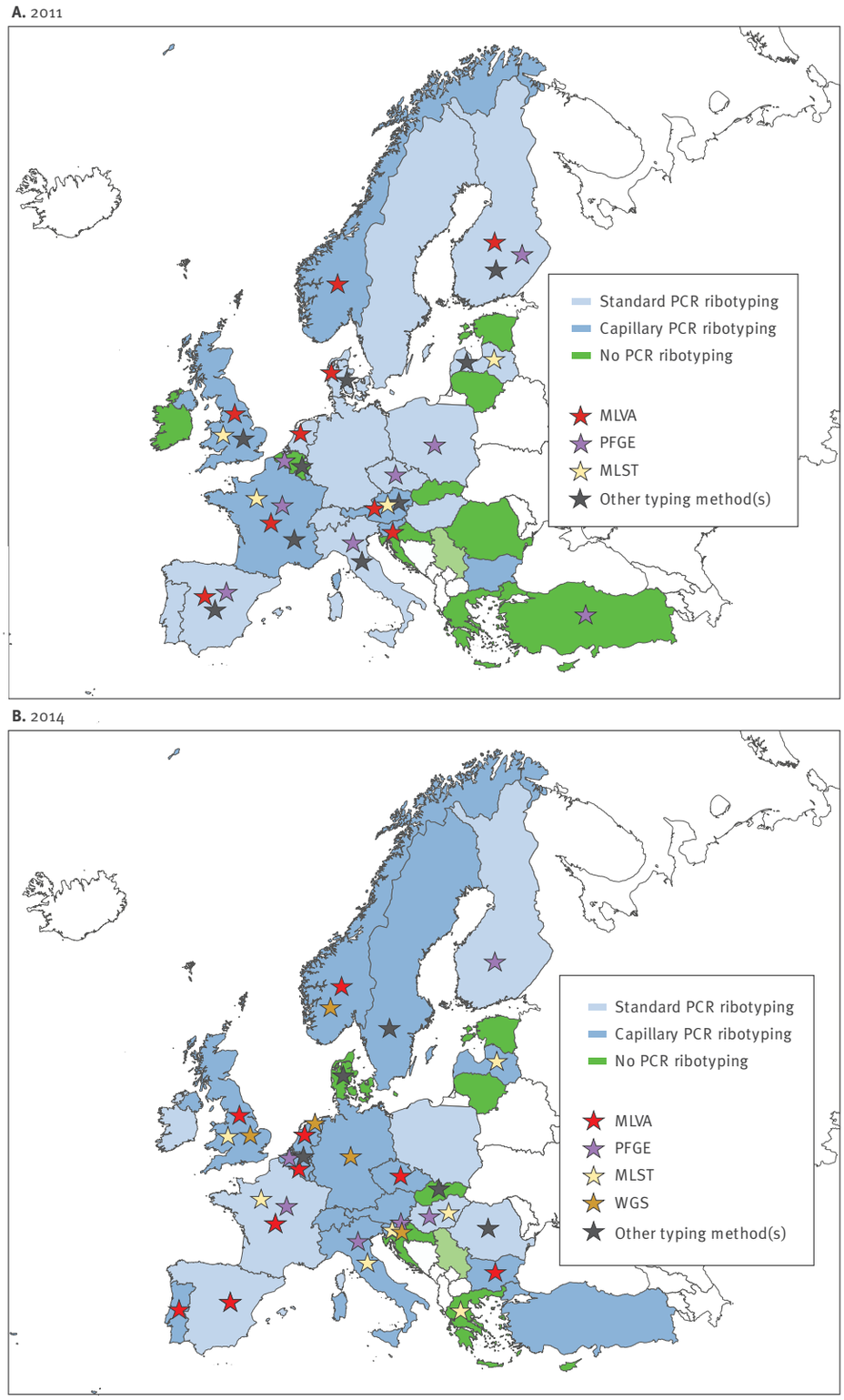


[a] Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lichtenstein, Lithuania, Luxembourg, the Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom (data were analysed separately for England, Northern Ireland, Scotland and Wales, but counted as one country). No data were available for Iceland.

[b] Seven countries reported other changes in national laboratory diagnostics: Slovenia was developing new national guidelines for CDI at the time of the second survey; Romania started a national surveillance study in 2014; Spain published an opinion document on CDI [32]; Slovakia was in the process of implementing new diagnostic methods due to an increased interest in CDI; in Cyprus, the central diagnostic laboratory for C. difficile implemented a two-step diagnostic algorithm; in Finland, CDI diagnostics were subcontracted to laboratory consortia that applied nucleic acid amplification tests more often; and Hungary relocated its national reference laboratory to expand its laboratory capacity but still had limited resources.

Figure 3

Clostridium difficile typing methods available in countries that participated in the European *Clostridium difficile* Infection Surveillance Network (ECDIS-Net) project in 2011 (n = 31)[a] and 2014 (n = 32)[a]



PFGE: pulsed-field gel electrophoresis; MLST: multilocus sequence typing; MLVA: multilocus variable-number tandem repeat analysis; WGS: whole genome sequencing.

Other typing methods used in 2011 were: tcdC typing (Austria, Belgium, Finland, France, Italy, Latvia, Luxembourg (not shown), Spain, United Kingdom - Northern Ireland only), repetitive-element PCR (Belgium, Spain), toxinotyping (Italy, Spain), tandem repeat sequence typing (Denmark) and pathogenicity locus (PaLoc) multiplex PCR (Finland).

Other typing methods used in 2014 were: tcdA/B (Belgium, Romania, Slovakia), CDT (Belgium, Slovakia), tcdC (Belgium), Δ117TcdC (Slovakia), and GyrAΔ detection (Belgium) detection, tandem repeat sequence typing (Denmark), and high molecular weight typing by MALDI-TOF (Sweden).

a In 2011, 31 countries responded: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lichtenstein, Lithuania, Luxembourg, the Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom (not including Wales). No data were available for Iceland. In 2011, Serbia did not participate in the European Clostridium difficile Infection Surveillance Network (ECDIS-Net) project. In 2014, Serbia participated in the ECDIS-Net project and responded to the 2014 questionnaire, as did Wales, and so the number of responding countries in 2014 was 32.

Source of map: FreeVectorMaps.com (http://freevectormaps.com).

Table 1

Criteria for categorisation of *Clostridium difficile* infection diagnostic algorithms, survey of European countries participating in the European *Clostridium difficile* Infection Surveillance Network (ECDIS-Net) project, 2011 (n = 31)[a] and 2014 (n = 26)[a]

Categorisation of CDI diagnostics		CDI diagnostic algorithm	
Screening test		Confirmatory test	
Optimal[b]	1[c]	NAAT	EIA toxin detection
	2–3[c]	GDH EIA and toxin detection	NAAT or toxigenic culture
Acceptable[b]	4–5[c]	GDH EIA detection	NAAT or toxigenic culture
	6[c]	NAAT	None
Incomplete[b]	7–10[c]	All other algorithms	

CDI: *Clostridium difficile* infection; EIA: enzyme immunoassay; GDH: glutamate dehydrogenase; NAAT: nucleic acid amplification test.

[a] Laboratories in 31 countries responded to the 2011 survey: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lichtenstein, Lithuania, Luxembourg, the Netherlands, Norway, Poland, Portugal, Romania, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom (not including Wales). Serbia did not participate in the European *Clostridium difficile* Infection Surveillance Network (ECDIS-Net) project in 2011. No laboratories in Slovakia and Wales were invited to participate by ECDIS-Net national coordinators in 2011. Laboratories in 26 countries responded in 2014 (no data from laboratories in Croatia, Iceland, Latvia, Slovenia and Switzerland).

[b] Categorisation of CDI diagnostic algorithms in the second survey, in 2014 [21].

[c] Corresponding CDI diagnostic algorithms in the second survey, in 2014 [21].

CDI testing. ESCMID recommended performing CDI testing not only upon request of a medical doctor, but also based on other indications such as the 'three-day rule', i.e. diarrhoea after three days of hospitalisation or when diarrhoea develops after antibiotic use [6,13].

The type of diagnostic algorithm applied influences not only clinical care [14], but also CDI surveillance's sensitivity and specificity [3,14,15]. However, a consensus on when and how to test for CDI has not been established among reference and local laboratories.

Additionally, typing of *C. difficile* to understand its local or wider transmission remains non-standardised in Europe [16,17]. Numerous typing methods have become available for routine use in the last 30 years. For *C. difficile*, these include methods that use restriction enzymes (e.g. restriction endonuclease analysis, pulsed-field gel electrophoresis (PFGE)), PCR amplification of housekeeping genes (e.g. multilocus sequence typing (MLST)), of repetitive elements (repetitive-element PCR, multilocus variable-number tandem repeat analysis (MLVA)), of the pathogenicity locus (e.g. toxinotyping) or of 16S-23S rRNA intergenic spacer regions (e.g. PCR ribotyping) [16,18]. Whole genome sequencing, with its ultimate discriminatory power, can already be used for in-depth analysis of evolutionary patterns [19]. Nevertheless, PCR ribotyping still remains the standard typing method in Europe as it involves relatively simple technology and its low costs permits widespread application [16,18].

In 2010, the European Centre for Disease Prevention and Control (ECDC) launched the European *C. difficile* Infection Surveillance Network (ECDIS-Net) project, an initiative to enhance and harmonise laboratory diagnostic and typing capacity for CDI, and to support surveillance of CDI in Europe. The project consortium consisted of a team of experts involved in the first European multicountry surveillance study performed in 2008 [20]. Between 2010 and 2014, the ECDIS-Net project developed standard operating procedures for *C. difficile* culturing and PCR ribotyping, implemented a reference nomenclature database and compiled a set of reference strains to standardise PCR ribotyping. National reference laboratories were invited to participate in a workshop for culturing and typing of *C. difficile* and participated in an external quality assessment exercise.

The study presented here measured changes in capacity for diagnostic testing for CDI and typing of *C. difficile* isolates in Europe between 2011 and 2014, using surveys of European local laboratories and national coordinators participating in the ECDIS-Net project. Additionally, we aimed to obtain insight into barriers to optimal CDI laboratory diagnostics, to inform further activities of ECDC and of the ESCMID Study Group for C. *difficile* (ESGCD) in this field.

Methods

Study design

The Dutch National Reference Laboratory for *C. difficile* (Leiden University Medical Centre, Leiden, and the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands) coordinated data collection in 2011 and in 2014 by cross-sectional surveys among two target groups: (i) local microbiology laboratories, in order to evaluate changes in routine laboratory diagnostics; and (ii) national coordinators, i.e. representatives of national or regional reference laboratories nominated by competent bodies for surveillance on the request of ECDC, to evaluate national changes in diagnostic and typing capacity for *C. difficile*. In 2011 and 2014, 32 and 33 countries participating in the ECDIS-Net project were invited to take part in

the survey, respectively (in 2011, Serbia did not participate in ECDIS-Net). All surveys are available online [21].

Selection

There was no European register of microbiology laboratories to use for random sampling. Therefore, ECDIS-Net national coordinators were requested to invite a representative sample of the local clinical microbiology laboratories (about 10%) in each country to participate in the survey. In Austria and Norway, the laboratories were selected by random sampling; all other countries used non-random convenience sampling [22]. Selected laboratories were emailed an initial survey in October 2011: some laboratories replied in 2012. All respondents to the initial survey received a follow-up survey in June 2014.

Data collection

Data were collected through a centralised web-based system (Questback, New York, United States). In 2011, the initial survey contained questions on several aspects of local routine diagnostics, including indications for undertaking CDI diagnostics and methodologies. Laboratories were requested to report the type of screening test primarily used for CDI diagnostics and confirmatory test (if applicable). For both, they could report more than one test. In 2014, the follow-up survey listed 10 diagnostic algorithms each designated as either 'optimal', 'acceptable' or 'incomplete' (Table 1). Laboratories were requested to estimate the percentage of samples that had been tested according to each algorithm listed, or to describe their usual diagnostic algorithm and estimate the corresponding percentage. The categorisation of CDI diagnostic algorithms was made by some of the ECDIS-Net experts who were also involved in revising the ESCMID diagnostics guidelines for CDI [6]. Algorithms designated as optimal had high sensitivity and specificity (not specifically defined), detection of free toxins in faeces and a rapid turnaround time [23]. Acceptable algorithms met the same criteria but without detecting free toxins in faeces. Any other algorithm was designated as incomplete. The 2014 follow-up survey additionally contained questions on barriers to apply optimal or acceptable diagnostic algorithms and changes in the indications for sending samples for CDI diagnosis by medical doctors.

Data analysis

To allow comparison, data on diagnostics from the 2011 initial survey were distributed into the three categories of diagnostic algorithms defined in 2014. For each local laboratory, CDI diagnostics, i.e. CDI testing practices, were considered optimal if more than 80% of the samples followed an optimal diagnostic algorithm, and acceptable if more than 80% of the samples followed either an optimal or acceptable algorithm. CDI diagnostics of all other algorithms were considered incomplete. When a laboratory reported a three-step algorithm by applying a third diagnostic test when the screening and confirmatory tests were contradictory, this algorithm was allocated to the best-matching two-step algorithm. Changes in local laboratory diagnostic capacity were evaluated by the McNemar's test [24], and changes in the use of optimal, acceptable and incomplete algorithms in 2011 and 2014 were evaluated by a Bowker test for symmetry [24]. A sensitivity analysis was performed using two assumptions on missing data in 2014, i.e. CDI diagnostics one category inferior (Table 1) than in 2011 and CDI diagnostics one category superior than in 2011. Data were analysed using IBM SPSS statistics 20 (SPSS Inc., Chicago, United States).

Survey of ECDIS-Net national coordinators

Data collection and analysis

All ECDIS-Net national coordinators received an initial survey in May 2011 and a follow-up survey in June 2014. Both surveys contained questions on national typing capacity (defined as any laboratory in the country performing typing) and on molecular typing methods, asking which were available in their country from a list of common methods [18].

Results

Local laboratory capacity

Participants

Questionnaires on local diagnostic and typing capacity for CDI were completed by 126 (61%) of 206 laboratories in 2011–12 and by 84 (67%) of these same 126 laboratories in 2014 (Table 2). A total 124 (98%) of the 126 responding laboratories in 2011–12 provided microbiological services to hospitals, of which 103 (83%) served at least one university, secondary or tertiary care hospital. In addition, 66 (53%) provided microbiological services to long-term care facilities, of which 45 provided services to nursing homes. Furthermore, 65/124 (52%; data were missing for two laboratories) provided medical services to other healthcare services (e.g. general practitioners). In 2011 and 2014, 120/126 (95%) and 83/84 laboratories (99%, among responders to both questionnaires; $p = 0.50$), respectively, reported that they performed CDI laboratory diagnostics.

Indications for *Clostridium difficile* infection diagnostics

The indications for CDI diagnostics reported in 2011 are listed in Figure 1. In 2014, a change of indications for sending samples for CDI diagnosis by medical doctors was observed; 16 (19%) of 83 laboratories reported that one or two changes had occurred since 2011. Several laboratories introduced the use of Bristol stool scores to assess stool consistency for sample selection ($n = 5$). Also, patient populations that were previously not monitored for CDI (e.g. outpatients, high-risk populations) were later explicitly included in protocols ($n = 3$) and awareness and recognition of CDI among clinicians had improved ($n = 5$). Other improvements of sample selection were also reported ($n = 5$), i.e. application of guidelines for sample selection ($n = 3$) and/or the three-day rule, i.e. diarrhoea after three days

Table 2

Response of participating European countries to local laboratory (n = 31 and n = 26, respectively) and national/subnational surveys (n = 31 and n = 32, respectively) on *Clostridium difficile* infection diagnostic and typing capacity, 2011 and 2014

Country	Number of laboratories that responded to local questionnaire / number invited		Replied to national questionnaire[a]	
	2011	2014	2011	2014
Austria	4/8	2/4	Yes	Yes
Belgium	4/9	4/4	Yes	Yes
Bulgaria	7/7	2/7	Yes	Yes
Croatia	2/4	0/2	Yes	Yes
Cyprus	3/3	3/3	Yes	Yes
Czech Republic	9/11	7/9	Yes	Yes
Denmark	3/3	1/3	Yes	Yes
Estonia	2/2	1/2	Yes	Yes
Finland	3/3	2/3	Yes	Yes
France	5/37	2/5	Yes	Yes
Germany	5/7	5/5	Yes	Yes
Greece	3/3	2/3	Yes	Yes
Hungary	8/8	8/8	Yes	Yes
Iceland	1/1	0/1	No	No
Ireland	3/5	2/3	Yes	Yes
Italy	13/14	8/13	Yes	Yes
Latvia	2/3	0/2	Yes	Yes
Lichtenstein	1/1	1/1	Yes	Yes
Lithuania	3/3	2/3	Yes	Yes
Luxembourg	2/6	1/2	Yes	Yes
Netherlands	4/6	3/4	Yes	Yes
Norway	9/13	4/9	Yes	Yes
Poland	6/6	4/6	Yes	Yes
Portugal	4/5	4/4	Yes	Yes
Romania	4/6	3/4	Yes	Yes
Serbia[b]	NA	NA	NA	Yes
Slovakia[c]	NA	NA	Yes	Yes
Slovenia	1/3	0/1	Yes	Yes
Spain	3/5	2/3	Yes	Yes
Sweden	2/3	2/2	Yes	Yes
Switzerland	1/1	0/1	Yes	Yes
Turkey	2/7	2/2	Yes	Yes
UK-England	2/6	2/2	Yes	Yes
UK-Northern Ireland	1/3	1/1	Yes	Yes
UK-Scotland	4/4	4/4	Yes	Yes
UK-Wales[c]	NA	NA	No	Yes
Total	**126/206**	**84/126**	**31**	**32**

NA: not applicable; UK: United Kingdom.

[a] For the UK, data were analysed separately for England, Northern Ireland, Scotland and Wales, but the UK was counted as one country.

[b] Serbia did not participate in the European *Clostridium difficile* Infection Surveillance Network (ECDIS-Net) project in 2011.

[c] No laboratories in Slovakia and Wales were invited to participate by ECDIS-Net national coordinators.

of hospitalisation (n = 1), and unspecified attempts to improve sample selection (n = 1).

C. difficile infection diagnostics

In 2011, 17 (14%) of 120 laboratories had optimal CDI diagnostics, 12 (10%) acceptable diagnostics and 91 (76%) incomplete diagnostics (Table 3). Incomplete algorithms included use of EIA toxin detection for screening with or without a confirmatory test, or a combination of EIA GDH and toxin detection without other tests for confirmation. Among laboratories responding to both the 2011 and 2014 surveys and that performed CDI diagnostics at both time-points (n = 81), the percentage of laboratories with optimal CDI diagnostics

increased from 19% to 46% and that with acceptable CDI diagnostics from 10% to 15% while the percentage of laboratories with incomplete CDI diagnostics decreased from 72% to 40% ($p < 0.001$; Table 3). Two laboratories without any diagnostics in 2011 had optimal and incomplete CDI diagnostics, respectively, in 2014.

Sensitivity analysis

Laboratories with optimal CDI diagnostics in 2011 were more likely to respond to the 2014 survey (15/17) compared with those with acceptable (8/12) or incomplete diagnostics (58/91). Under the negative assumption that all non-responding laboratories in 2014 applied CDI diagnostics one category inferior in 2014 compared with that of 2011, the percentage of laboratories with optimal diagnostics would have increased from 14% to 31%, that with acceptable diagnostics would have increased from 10% to 12%, and that with incomplete diagnostics would have decreased from 76% to 58% between 2011 and 2014 ($p < 0.001$). Conversely, if all non-responding laboratories had CDI diagnostics one category superior in 2014 compared with 2011, the percentage of laboratories with optimal diagnostics would have increased from 14% to 36%, that with acceptable diagnostics would have increased from 10 to 38%, and that with incomplete diagnostics would have decreased from 76 to 27% between 2011 and 2014 ($p < 0.001$).

Barriers to optimal/acceptable diagnostics for *C. difficile* infection

Barriers to applying optimal or acceptable algorithms were examined in 2014. Of the 33 laboratories with incomplete CDI diagnostics, 17 indicated that materials or tests were too costly, six indicated receiving insufficient reimbursement for tests from insurers and five had insufficient availability of trained staff. Of the 50 laboratories that had optimal or acceptable CDI diagnostics, 10 also indicated that materials or tests were too costly, seven indicated receiving insufficient reimbursement from insurers and five had insufficient availability of trained staff. Ten laboratories that responded in 2014 indicated that they disagreed with the project's designations of the CDI diagnostic algorithms as optimal, acceptable or incomplete.

National/subnational capacity

Participating countries

The national coordinators of 31 and 32 countries responded to the national survey in 2011 and 2014, respectively (Table 2). Data were collected separately for the four countries within the United Kingdom (UK), i.e. England, Northern Ireland, Scotland and Wales, but the UK was counted as one country.

Changes in national diagnostic capacity

In 2014, eight of the 32 responding countries (France, Germany, Greece, Latvia, Luxembourg, Sweden, Switzerland, Turkey) reported no change in national/subnational laboratory diagnostics for CDI. Conversely, 24 countries reported one or more changes in national/subnational laboratory diagnostics for CDI since 2011 (Figure 2). Specifically, 16 countries had experienced a change in availability of commercial diagnostic tests (Bulgaria, Czech Republic, Estonia, Hungary, Ireland, Italy, Lichtenstein, Lithuania, the Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovenia, UK), 10 countries had new or revised guidelines for CDI diagnostics (Austria, Czech Republic, Denmark, Estonia, Hungary, Ireland [25], Italy, Lithuania, Romania, UK) and three countries had changes in relevant legislation (Hungary, Poland, Romania). Three countries (Belgium, Croatia, Czech Republic) had implemented changes in reimbursement policies for diagnostic tests. Greece had limited access to and reimbursement of materials in both 2011 and 2014. In 2012, the UK implemented 'harmonised' diagnostics using GDH screening (or NAAT) and EIA toxin detection (or CCA) in all its laboratories [26].

C. difficile national typing methods

The capacity for various *C. difficile* typing methods in participating countries in 2011 and 2014 is depicted in Figure 3. The number of countries able to perform any method of typing increased from 22/31 countries in 2011 to 26/32 countries in 2014. Only six countries (Croatia, Cyprus, Estonia, Lichtenstein, Lithuania, Serbia) reported that they did not have any national typing capacity in 2014 (none of these countries had typing capacity in 2011); however, Lichtenstein sent samples to another country (Austria) for typing.

Several typing methods were implemented by the countries (Figure 3). PCR ribotyping (either capillary-based or conventional agarose gel-based), the current European standard for *C. difficile* typing, was available in 20/31 countries in 2011 and in 23/32 countries in 2014. Two of the countries that acquired ribotyping capacity (Ireland and Romania) use it for national surveillance. Capillary PCR ribotyping was applied by 7/31 countries in 2011 and by 16/32 countries in 2014. In 2014, nine of the 32 participating countries applied MLVA, six PFGE and seven MLST. In 2014, whole genome sequencing was available in Germany, the Netherlands, Norway, Slovenia and England.

Some countries reported specific changes in national molecular typing capacity between 2011 and 2014. Greece, which previously did not have typing capacity, introduced MLST in January 2014. At the time of the 2014 survey, Estonia was capable of ribotyping for research projects, although there were no such projects. Turkey performed PCR ribotyping but lacked software to analyse the data. Denmark stopped using PCR ribotyping and only applied tandem repeat sequence typing. Hungary reported limited typing capacity for financial reasons although PCR ribotyping remained available at the national reference laboratory. Finland restricted the indications for ribotyping to severe CDI or outbreaks, which unintentionally caused many

Table 3

Laboratories participating in the European *Clostridium difficile* Infection Surveillance Network (ECDIS-Net) project according to their diagnostics category, 2011 (n = 120)a and 2014 (n = 81)[a]

Categorisation of CDI diagnostics[b]	All laboratories that provided data	Only laboratories that provided data in both 2011 and 2014 surveys	
	2011	2011	2014[c]
	n (%)	n (%)[d]	n (%)[d]
Optimal	17 (14)	15 (19)	37 (46)
Acceptable	12 (10)	8 (10)	12 (15)
Incomplete	91 (76)	58 (72)	32 (40)
Total	**120 (100)**	**81 (100)**	**81 (100)**

CDI: *Clostridium difficile* infection.

[a] Laboratories in 31 countries responded to the 2011 survey: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lichtenstein, Lithuania, Luxembourg, the Netherlands, Norway, Poland, Portugal, Romania, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom (not including Wales). Serbia did not participate in the European *Clostridium difficile* Infection Surveillance Network (ECDIS-Net) project in 2011. No laboratories in Slovakia and Wales were invited to participate by ECDIS-Net national coordinators in 2011. Laboratories in 26 countries responded in 2014 (no data from laboratories in Croatia, Iceland, Latvia, Slovenia and Switzerland).

[b] CDI diagnostics were considered 'optimal' if>80% of the samples followed an 'optimal' testing algorithm, and 'acceptable' if>80% of the samples followed either an 'optimal' or 'acceptable' testing algorithm. CDI diagnostics of all other laboratories were considered 'incomplete'. The diagnostic algorithms are described in Table 1.

[c] Two laboratories that did not perform CDI laboratory diagnostics in 2011 were not included. These laboratories indicated in the 2014 questionnaire that they used optimal and incomplete CDI diagnostics, respectively.

[d] The percentages in this column do not add up to 100 due to rounding.

laboratories to stop all culturing and/or sending isolates for typing.

Discussion

This study assessed changes in diagnostic testing and typing capacity for CDI in Europe between 2011 and 2014, using surveys of European local laboratories and of national coordinators participating in the ECDIS-Net project. Virtually all participating local laboratories had implemented CDI diagnostics in 2011 and 2014, compared with 88% (186/212) of the local laboratories investigated in eight European countries in 2003 [27]. The percentage of laboratories with optimal CDI diagnostics increased from 19% to 46%, and that with acceptable diagnostics increased from 10% to 15%. Importantly, the ESCMID-recommended two-step diagnostic algorithm [6] became more common. Nevertheless, we still observed a considerable variation in CDI diagnostics within and between European countries, in line with another European study with 482 participating hospitals in 2011–13 [3]. This variation in diagnostics can substantially affect CDI incidence rates obtained by surveillance [15,28]. Our survey showed that suboptimal CDI diagnostics may result from, for example, financial restrictions or limited availability of trained staff. As a consequence of the disagreement by a sizable minority of laboratories with the designation of diagnostic algorithms, the ESGCD undertook to revise its diagnostic guidelines [6] and propose an algorithm that can also be implemented in laboratories with limited numbers of trained staff and limited financial resources. These revised guidelines will be published in 2016 on behalf of ESCMID.

Among countries having national guidelines available, the UK was the only one that had succeeded in harmonising CDI diagnostics, by recommending a single two-test diagnostic algorithm ('comprising a GDH EIA (or NAAT/PCR) followed by a sensitive toxin EIA') [3,26]. The recommendations in the UK Department of Health guidance were supported by local study data and inclusion of frequently asked questions to allay objections of the laboratories to implementing the proposed diagnostic algorithms [26]. Furthermore, the diagnostics guidance was one of many *C. difficile*-related activities in the UK, for example, implementation of mandatory CDI reduction targets with financial penalties for national health services [29]. There probably are two possible ways to optimise testing: either to promote one national diagnostic algorithm or to promote the use of optimal testing strategies by local laboratories. However, the proposed algorithm in the UK was not fully compliant with the designation of diagnostic algorithms as optimal in this survey, highlighting the need for further discussion among experts to reach a consensus. Another example is Spain, where several national studies and meetings were organised [30,31] that resulted in an opinion document to enhance optimal diagnostics for CDI [32]. We hope that the national reference laboratories that participated in the ECDIS-Net project will follow these examples and promote optimal diagnostics for CDI and its implementation in local laboratories.

Typing capacity

Between 2011 and 2014, PCR ribotyping capacity and capillary PCR ribotyping increased among the participating countries. Capillary PCR ribotyping was validated in 2012–14 by four reference laboratories in England, the Netherlands, the United States and Canada, identifying a 98% consensus (195/200 cases tested) between the laboratories, which indicated the

method's suitability for standardised CDI surveillance [17].

We assume that ECDIS-Net activities during 2012–14, including a training programme for *C. difficile* PCR ribotyping, contributed to the increased PCR ribotyping capacity. For example, Romania joined the training programme in 2012 and received a set of reference strains from the ECDIS-Net project and is now able to apply PCR ribotyping in their national surveillance. Poland reported having started their first national surveillance programme, stimulated by ECDIS-Net activities in 2012 [33]. A few countries (Hungary, Italy, Slovenia) had national surveillance under development at time of the 2014 survey. Despite these positive trends, our study also indicates that some European reference and local laboratories are affected by limited resources and budget reductions, which hamper implementation and technical improvements of molecular typing methods.

Limitations

This study has several limitations including the small, non-random selection of local laboratories for both surveys and the moderate response rate, limiting the degree to which conclusions can be extrapolated to all European microbiological laboratories. The representativeness of the invited and participating laboratories could not be assessed due to the absence of a suitably complete European register. Laboratories with better CDI diagnostics may have been more likely to participate in the original and follow-up surveys, leading to an overestimation of the number of laboratories with optimal CDI diagnostics in Europe. Additionally, the categorisation of CDI diagnostic algorithms into three levels, although made through a series of consultations with a team of international experts from the ECDIS-Net project, was based on expert opinion and some subjectivity cannot be excluded. Also, although the 2014 questionnaire for local laboratories requested quantitative data on the percentage of tests that followed each algorithm on a provided list, as the list had the subheadings 'optimal', 'acceptable' and 'incomplete', it is possible that those responding overestimated the proportion of desirable answers. We estimate that this reporting bias was minimal as for almost all laboratories, just one algorithm was used.

Conclusions

We conclude that the ECDIS-Net project laid the foundations for Europe-wide surveillance of CDI, although increased use of optimal diagnostic algorithms should be promoted, taking into consideration the limited resources and budget cuts in several European countries. The ESGCD revised the ESCMID diagnostics guidelines for CDI, which, once published, should contribute to standardisation of CDI diagnostics at local and national level in Europe. Typing capacity for CDI in Europe was acceptable overall; however, an internationally standardised capillary PCR ribotyping protocol is now available [17] and requires further implementation in European countries. We would recommend that these important steps are considered as part of the integration of *C. difficile* molecular typing data in The European Surveillance System (TESSy), within the ECDC-coordinated Europe-wide CDI surveillance (since 1 January 2016) [34].

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Conflict of interest

Sofie M van Dorp, Daan W Notermans, Jeroen Alblas, Petra Gastmeier, Katiusha Ivanova, Fidelma Fitzpatrick, Trefor Morris, Pete Kinross, Carl Suetens: none declared.

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Authors' contributions

EK and MW were leaders of the ECDIS-Net project. DN was the leader of this project and coordinated data collection in 2011. SD coordinated data collection in 2014, analysed the data, and wrote the manuscript together with DN, EK and PK. Questionnaires were developed by DN, PK, CS, SD, FB and EK. JA adapted the web-system for data collection. All co-authors were participants in the project consortium, contributed to data collection and reviewed the manuscript.

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Standardised surveillance of *Clostridium difficile* infection in European acute care hospitals: a pilot study, 2013

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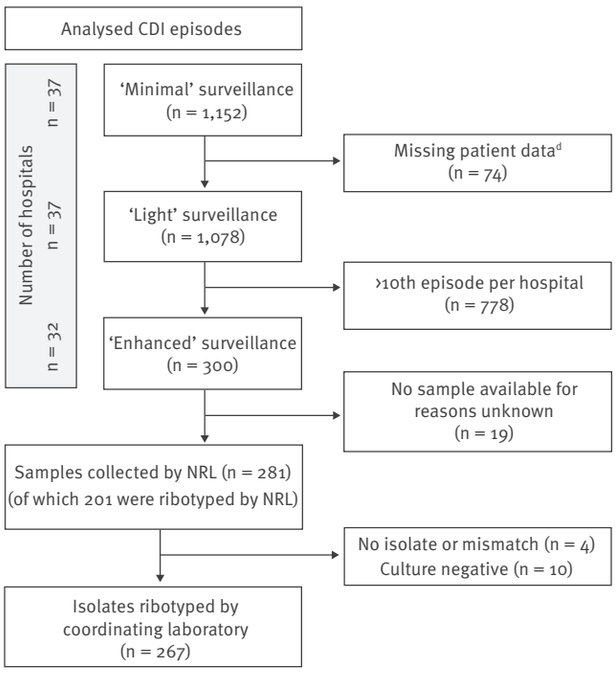
Clostridium difficile infection (CDI) remains poorly controlled in many European countries, of which several have not yet implemented national CDI surveillance. In 2013, experts from the European CDI Surveillance Network project and from the European Centre for Disease Prevention and Control developed a protocol with three options of CDI surveillance for acute care hospitals: a 'minimal' option (aggregated hospital data), a 'light' option (including patient data for CDI cases) and an 'enhanced' option (including microbiological data on the first 10 CDI episodes per hospital). A total of 37 hospitals in 14 European countries tested these options for a three-month period (between 13 May and 1 November 2013). All 37 hospitals successfully completed the minimal surveillance option (for 1,152 patients). Clinical data were submitted for 94% (1,078/1,152) of the patients in the light option; information on CDI origin and outcome was complete for 94% (1,016/1,078) and 98% (294/300) of the patients in the light and enhanced options, respectively. The workload of the options was 1.1, 2.0 and 3.0 person-days per 10,000 hospital discharges, respectively. Enhanced surveillance was tested and was successful in 32 of the hospitals, showing that *C. difficile* PCR ribotype 027 was predominant (30% (79/267)). This study showed that standardised multicountry surveillance, with the option of integrating clinical and molecular data, is a feasible strategy for monitoring CDI in Europe.

Introduction

After recognition of European outbreaks of *Clostridium difficile* infections (CDIs) associated with the emergence of PCR ribotype 027/NAP1 in 2005, CDI surveillance at country level was encouraged by the European Centre for Disease Prevention and Control (ECDC) [1]. In 2008, an ECDC-supported European CDI survey (ECDIS) identified large intercountry variations in incidence rates and distribution of prevalent PCR ribotypes, with the outbreak-related PCR ribotype 027 being detected in 5% (range: 0–26) of the characterised isolates [2]. The surveillance period was limited to one month and the representation of European hospitals was incomplete; however, this has been the only European (comprising European Union (EU)/European

Figure 1

Data collection in the pilot study for standardised surveillance[a] of *Clostridium difficile* infection in 37 acute care hospitals in 14 European countries[b], 13 May–1 November 2013[c]



CDI: Clostridium difficile infection.

[a] Three surveillance options were tested: 'minimal' (aggregated hospital data), 'light' (including patient data for CDI cases) and 'enhanced' (including microbiological data on the first 10 CDI episodes per hospital).

[b] Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, the Netherlands, Norway, Poland, Romania, Serbia, United Kingdom (Scotland only). Enhanced surveillance including PCR ribotyping was carried out by Austria, Belgium, Finland, France, Germany, Hungary, the Netherlands, Norway, Poland and United Kingdom (Scotland only); Denmark, Romania and Serbia participated in enhanced surveillance, but did not perform PCR ribotyping at the national reference laboratory or appointed study laboratory.

[c] Three-month assessment during this time period.

[d] Clincial patient data missing, for reasons unknown.

Economic Area (EEA) and EU candidate countries) CDI surveillance study. The authors highlighted the need for national and European surveillance to control CDI. Yet, European countries were found to have limited capacity for diagnostic testing, particularly in terms of standard use of optimal methods and absence of surveillance protocols and a fully validated, standardised and exchangeable typing system for surveillance and/or outbreak investigation.

As of 2011, 14 European countries had implemented national CDI surveillance, with various methodologies [3]. National surveillance systems have since reported a decrease in CDI incidence rate and/or prevalence of PCR ribotype 027 in some European countries [4-8]. However, CDI generally remains poorly controlled in Europe [9], and PCR ribotype 027 continues to spread in eastern Europe [10-12] and globally [13].

In 2010, ECDC launched a new project, the European *C. difficile* Infection Surveillance Network (ECDIS-Net), to enhance surveillance of CDI and laboratory capacity to test for CDI in Europe. The goal of ECDIS-Net was to establish a standardised CDI surveillance protocol suitable for application all over Europe in order to: (i) estimate the incidence rate and total infection rate of CDI (including recurrent CDI cases) in European acute care hospitals; (ii) provide participating hospitals with a standardised tool to measure and compare their own incidence rates with those observed in other participating hospitals; (iii) assess adverse outcomes of CDI such as complications and death; and (iv) describe the epidemiology of CDI concerning antibiotic susceptibility, PCR ribotypes, presence of tcdA, tcdB and binary toxins and detect new emerging types at local, national and European level.

The primary objectives of the present study were to: (i) test the pilot protocol for the surveillance of CDI in European acute care hospitals developed by ECDIS-Net (methodology, variables and indicators); (ii) assess the feasibility and workload of collecting the required hospital data, case-based epidemiological and microbiological data; and (iii) evaluate the quality of data collected, whether in the presence or absence of existing national CDI surveillance activities. A secondary aim was to assess the relationship between patient and microbiological characteristics and in-hospital outcome of CDI to confirm the added value of collecting detailed epidemiological and microbiological data on CDI at European level.

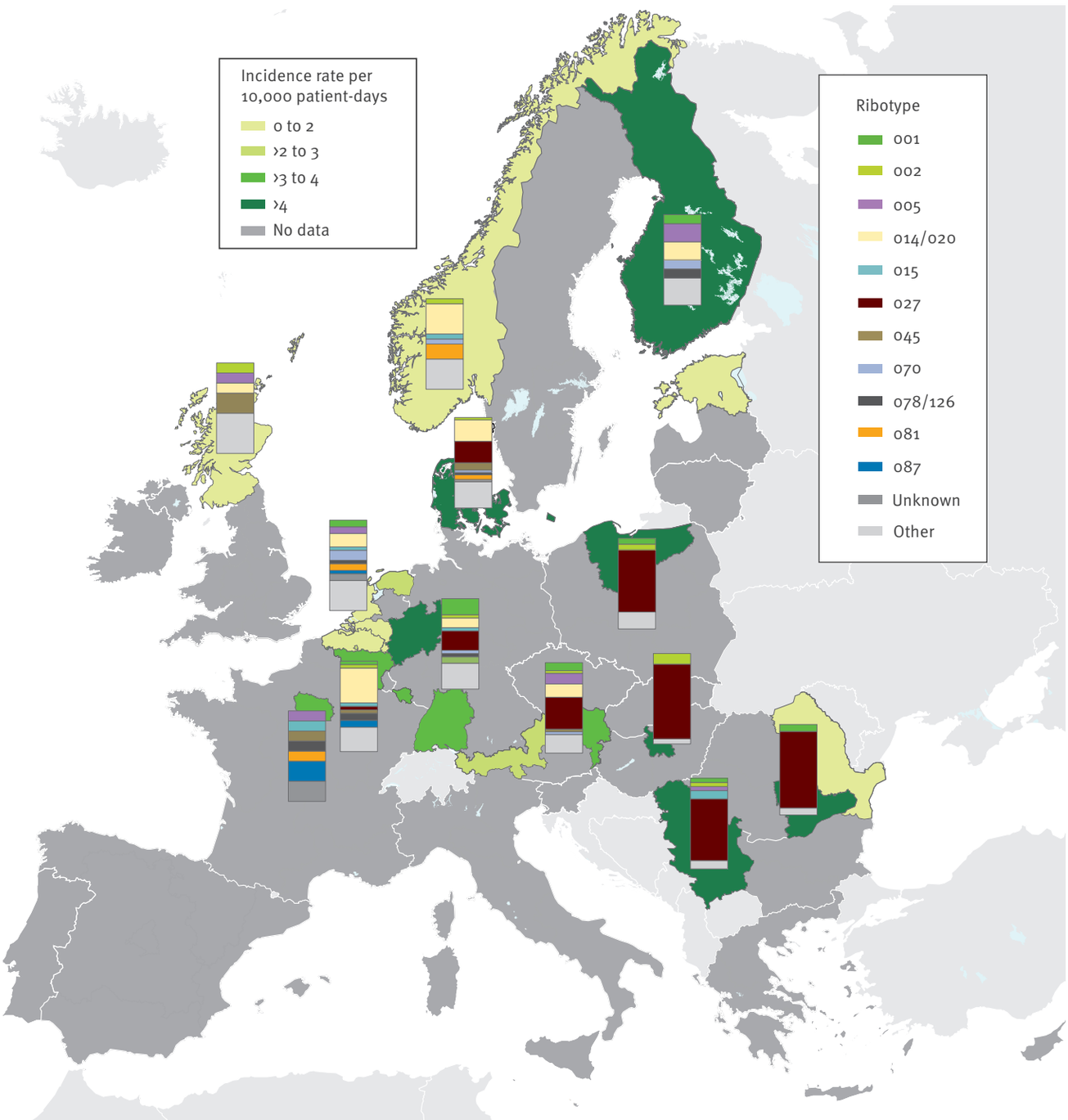
Methods

Study protocol and definitions

A pilot protocol for the surveillance of CDI in European acute care hospitals was developed by ECDIS-Net participants (epidemiologists and medical microbiologists from various European countries) and ECDC experts in 2012–13. The pilot protocol version 1.2 specified three options for surveillance: 'minimal', 'light' and 'enhanced' [14]. In the minimal surveillance, aggregated numerator and denominator data were gathered on all CDI cases. In the light surveillance, basic case-based epidemiological data were included (e.g. age, sex, date of hospital admission and of CDI onset, CDI origin, recurrent CDI) on all CDI cases. In the enhanced surveillance, additional epidemiological data (e.g. comorbidities scored by the McCabe score [15] and the Acute Physiology and Chronic Health Evaluation II (APACHE II) chronic health points [16], in-hospital deaths) and *C. difficile* isolates were collected for the

Figure 2

Incidence rate of healthcare-associated *Clostridium difficile* infection using 'minimal' surveillance[a], by region (n = 22)[b] and distribution of PCR ribotypes identified using enhanced surveillance, by European country (n = 13)[c], 13 May–1 November 2013[d]



CDI: Clostridium difficile infection; NUTS: nomenclature of territorial units for statistics.

The pilot study was based on a non-representative sample, thus the rates and distributions presented in this figure cannot be interpreted as being representative of any NUTS region.

[a] The 'minimal' surveillance option comprised aggregated hospital data; the 'enhanced' option included microbiological data on the first 10 CDI episodes per hospital.

[b] The NUTS 1 region indicates the geographical location of each participating hospital, rather than that of the hospital's catchment area. The incidence rate per 10,000 patient-days in each NUTS 1 region is the median for all hospitals that participated within that same region.

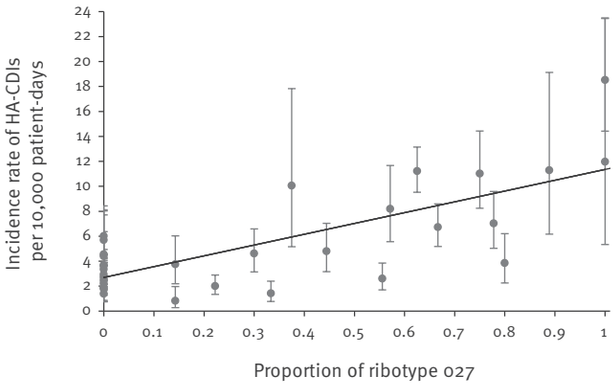
[c] The number of PCR ribotyped strains varied by country: Austria (34), Belgium (26), Denmark (38), Finland (10), France (9), Germany (28), Hungary (17), the Netherlands (27), Norway (18), Poland (16), Romania (13), Serbia (22) and United Kingdom (Scotland only) (9).

[d] Three-month assessment during this time period.

Source of map: FreeVectorMaps.com (http://freevectormaps.com).

Figure 3

Incidence rate of healthcare-associated *Clostridium difficile* infection in relation to the proportion of PCR ribotype 027 isolates, from 'enhanced' surveillance[a] in acute care hospitals in 13 European countries[b], 13 May–1 November 2013[c]



CA: community-associated; CDI: Clostridium difficile infection; CI: confidence interval; HA: healthcare-associated.

Whiskers indicate the 95% CI around the incidence rate of HA-CDI per 10,000 patient-days per hospital. The proportion of PCR ribotype 027 isolates correlated with the incidence rate (Spearman's rho: 0.64; 95% CI: 0.36–0.81).

[a] The 'enhanced' surveillance option included microbiological data on the first 10 CDI episodes per hospital.

[b] Austria, Belgium, Denmark, Finland, France, Germany, Hungary, the Netherlands, Norway, Poland, Romania, Serbia and United Kingdom (Scotland only).

[c] Three-month assessment during this time period.

first 10 episodes of CDI per hospital. Outcome was not followed up after discharge from the hospital.

The case definitions for CDI (Box) were based on recommendations for CDI surveillance, as proposed by ECDC and the United States Centers for Disease Control and Prevention (CDC) [1,17].

Patients were included as a CDI case if symptom onset occurred within the hospitals' surveillance period, or if the patient was admitted during the surveillance period with symptoms present. Infants (children below two years-old) with 'compelling clinical evidence for CDI' were also included.

Participants and study period

A total of 14 countries participated in this pilot study: they were selected by the project leaders given their various levels of ongoing surveillance activities and laboratory and typing capacity for CDI [18]. At the start of the ECDIS-Net project, nine countries (Austria, Belgium, Denmark, Finland, France, Germany, Hungary, the Netherlands and United Kingdom (Scotland only), hereafter referred to as UK-Scotland) had already implemented national surveillance of CDI; five countries (Estonia, Norway, Poland, Romania and Serbia) had not. ECDIS-Net participants identified a convenience sample of two to four acute care hospitals per country to test the pilot protocol for a three-month surveillance period between 13 May and 1 November 2013. Hospitals were encouraged, but not obligated, to test all surveillance options in the protocol and to involve both hospital infection control personnel and microbiology laboratory personnel in data collection. It was agreed that the actual location of participating hospitals would not be disclosed for reasons of confidentiality. We identified the proxy location of participating hospitals by mapping the median healthcare-associated CDI incidence rates obtained in this pilot study using the nomenclature of territorial units for statistics (NUTS) 1 regions [19] that contained at least one participating hospital.

Microbiological investigation

Local laboratories that serviced the participating hospitals used their own diagnostic procedures for CDI. Data on the algorithm used for CDI diagnosis was collected for each patient included in light surveillance. In the enhanced surveillance option, 10 *C. difficile* isolates (or stool samples, if there was no possibility of anaerobic culture at the local laboratory) from samples from the first 10 episodes of CDI per hospital were sent to the national reference laboratory or appointed study laboratory (collectively referred to as NRL) which performed PCR ribotyping and antimicrobial susceptibility testing, performed according to national procedures. Most NRLs used conventional agarose gel-based PCR ribotyping [3] (Finland, France, Hungary, Poland, the Netherlands and UK-Scotland), some used capillary-based PCR ribotyping [3] (Austria, Belgium and Germany). Denmark, Estonia, Romania and Serbia did not perform PCR ribotyping and for Norway, the PCR ribotyping method used was not reported. NRLs were requested to send all *C. difficile* isolates to the coordinating laboratory (Leiden University Medical Centre, the Netherlands), which completed and confirmed microbiological results. The presence of a glutamate dehydrogenase (GDH) gene specific for *C. difficile* was confirmed in the coordinating laboratory by an in-house PCR [20], followed by PCR ribotyping [21]. Toxin genes (*tcdA, tcdB, cdtA, cdtB*) were detected by multiplex PCR [22]. In vitro susceptibility to metronidazole, vancomycin, and moxifloxacin was determined by measuring minimum inhibitory concentrations (MICs) by an agar dilution method [23] and interpreted using epidemiological cut-off values from the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Isolates with a metronidazole MIC › 2 mg/L, a vancomycin MIC › 2 mg/L and moxifloxacin MIC › 4 mg/L were interpreted as resistant [24].

Data handling

Data were entered in a web-based system developed for the current study (by the Institute of Hygiene and

Box

Definitions for surveillance of *Clostridium difficile* infections

CDI case
A patient to whom one or more of the following criteria applies: 1. diarrhoeal stools or toxic megacolon AND a positive laboratory assay for C. difficile TcdA and/or TcdB in stools or a toxin-producing C. difficile organism detected in stool via culture or other means; 2. pseudomembranous colitis revealed by lower gastrointestinal endoscopy; 3. colonic histopathology characteristic of CDI (with or without diarrhoea) on a specimen obtained during endoscopy, colectomy or autopsy.
Recurrent CDI
An episode of CDI (return of diarrhoeal stools with a positive laboratory test after the end of treatment) > 2 weeks and ≤ 8 weeks following the onset of a previous episode (CDI cases with onset later than 8 weeks after the onset of a previous episode were included as new CDI cases).
Healthcare-associated case A case of CDI with onset of symptoms at least 48 hours following admission to a healthcare facility or with onset of symptoms in the community within 4 weeks following discharge from a healthcare facility.
Community-associated case
A case of CDI with onset of symptoms outside a healthcare facility or within 48 hours after admission to a healthcare facility, without residence in/discharge from a healthcare facility within the previous 12 weeks.
Complicated course of CDI CDI leading to any of the following: 1. admission to an intensive-care unit for treatment of CDI or its complications (e.g. for shock requiring vasopressor therapy); 2. surgery (colectomy) for toxic megacolon, perforation or refractory colitis; 3. death within 30 days after diagnosis if CDI is either a primary or contributing cause.

CDI : *Clostridium difficile* infection.

Source: [1,17].

Environmental Medicine, Charité Universitätsmedizin Berlin, Germany, in 2013) and were analysed with SPSS version 20.0 and Stata software version 12.1.

Statistical analysis and study endpoints

Primary endpoints

Variables and indicators

For all variables in each surveillance option, frequencies and proportions were calculated, as appropriate. Hospital median incidence rates for healthcare-associated (HA) CDI and recurrent CDI were calculated per 10,000 hospital discharges and per 10,000 patient-days using minimal surveillance protocol data. Dispersion around the median was described with the 25th and 75th percentile (interquartile range, IQR). We calculated 95% confidence intervals (CIs) for the incidence rates by Byar's approximation.

Feasibility and workload

Workload, defined as person-days per 10,000 hospital discharges required to complete each surveillance option, and feasibility were measured using a questionnaire distributed to all participants.

Data quality

Epidemiological data quality was primarily assessed by data completeness. This was estimated by comparing each hospital's minimal surveillance numerators (minimal option) with the number of available patient records (light option), and by calculating the proportion of patients for whom origin of the CDI (light option) and course of infection (enhanced option) were recorded, with less than 10% missing data being considered acceptable.

Microbiological data quality was assessed through comparison of each hospital's testing rate per 10,000 patient-days and percentage of positive tests. Additionally, all NRLs' ribotyping results obtained during the pilot study were compared with those of the coordinating laboratory. Additionally, in May 2013 and September 2014, participation in two external quality assessments was offered by Public Health England to all ECDIS-Net NRLs that performed typing. NRLs in nine of the participating countries took part; on each occasion, 10 *C. difficile* strains were sent to the same eight NRLs and the coordinating laboratory of this study.

Secondary endpoints

Relationships between the risk of a complicated course of CDI or all-cause in-hospital mortality in CDI cases (of any origin) and patient characteristics and microbiological results (as confirmed by the coordinating laboratory) were analysed by logistic regression. Correlations between incidence rates, testing rates and the proportion of PCR ribotype 027 were analysed by Spearman's rank test.

Table 1

Results from 'minimal' surveillance[a] of *Clostridium difficile* infection in 37 acute care hospitals in 14 European countries, 13 May–1 November 2013[b]

Country (number of hospitals)	Hospital discharges n	Patient-days n	CDI episodes included n	HA-CDIs n (%)	CA-CDIs and CDIs of unknown origin n (%)	Recurrent CDIs n (%)	Median incidence rate of HA-CDI	
							per 10,000 hospital discharges (range)	per 10,000 patient-days (range)
Austria (4)	56,773	307,721	117	88 (75)	16 (14)	13 (11)	15.8 (10.0–35.4)	3.2 (2.0–4.8)
Belgium (3)	20,434	140,603	53	32 (60)	13 (25)	8 (15)	17.7 (6.0–26.6)	2.7 (0.8–3.7)
Denmark (4)	60,572	182,888	171	120 (70)	25 (15)	26 (15)	17.7 (11.0–31.0)	5.3 (4.6–11.0)
Estonia (2)	18,293	133,790	18	16 (89)	1 (6)	1 (6)	8.6 (7.3–10.0)	1.2 (0.8–1.7)
Finland (3)	10,876	39,816	29	17 (59)	9 (31)	3 (10)	14.9 (12.2–20.8)	4.4 (2.6–6.5)
France (2)	9,608	64,203	46	31 (67)	9 (20)	6 (13)	26.7 (9.1–44.3)	3.8 (2.0–5.7)
Germany (3)	66,952	307,791	174	136 (78)	33 (19)	5 (3)	23.1 (16.2–28.2)	3.6 (3.4–6.7)
Hungary (2)	18,207	166,926	254	213 (84)	24 (9)	17 (7)	121.6 (111.5–131.8)	14.9 (11.2–18.5)
Netherlands (3)	20,388	123,507	43	29 (67)	11 (26)	3 (7)	10.5 (10.2–19.4)	1.9 (1.8–2.9)
Norway (2)	35,365	194,204	60	33 (55)	15 (25)	12 (20)	9.6 (8.5–10.8)	1.9 (1.4–2.5)
Poland (2)	15,182	86,771	69	65 (94)	4 (6)	0 (0)	42.6 (40.7–44.6)	7.6 (7.0–8.2)
Romania (2)	19,243	90,582	33	19 (58)	7 (21)	7 (21)	12.1 (8.0–16.5)	6.7 (1.4–12.0)
Serbia (3)	8,930	59,435	49	37 (76)	2 (4)	10 (20)	89.8 (22.0–131.8)	10.0 (3.9–11.3)
UK-Scotland (2)	26,554	94,942	36	16 (44)	13 (36)	7 (19)	5.3 (4.2–6.4)	1.4 (0.6–2.2)
Total (37)	**387,377**	**1,993,179**	**1,152**	**852 (74)**	**182 (16)**	**118 (10)**	**16.4 (4.2–131.8)**	**3.7 (0.6–18.5)**

CA: community-associated; CDI: *Clostridium difficile* infection; HA: healthcare-associated; UK-Scotland: United Kingdom (Scotland only).

The pilot study was based on a non-representative sample, thus the results presented cannot be interpreted as being representative of any participating country or of the European Union/European Economic Area.

[a] The 'minimal' surveillance option comprised aggregated hospital data.

[b] Three-month assessment during this time period.

Reporting

This study was reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Results

Participating hospitals

A total of 37 acute care hospitals from 14 European countries tested the minimal and light surveillance options for a three-month period between 13 May 2013 and 1 November 2013. Of the 37 acute care hospitals, 21 were tertiary care hospitals, 10 secondary care hospitals, five primary care hospitals and one was a specialised hospital for infectious and tropical diseases. A total of 36 hospitals included all wards; one hospital excluded a neonatal ward. Of the 37 participating hospitals, 32, from 13 countries, tested the enhanced option as well (Figure 1).

Minimal surveillance: incidence rate of *Clostridium difficile* infection

A total of 1,152 CDI episodes were recorded by minimal surveillance in 37 hospitals (Table 1).

After exclusion of recurrent episodes, the incidence rate of healthcare-associated CDI by hospital ranged from 4.2 to 131.8 per 10,000 hospital discharges (median: 16.4; IQR: 10.1–29.5) and from 0.6 to 18.5 per 10,000 patient-days (median: 3.7; IQR: 2.0–6.6). The incidence rate of recurrent CDI varied between 0 and 118.6 per 10,000 hospital discharges (median: 2.0; IQR: 0.2–5.2) and between 0 and 9.0 per 10,000 patient-days (median: 0.3; IQR: 0.04–1.2).

Light surveillance: patient characteristics and diagnostics

Patient data were submitted for 1,078 CDI episodes in 37 hospitals (Figure 1). Most CDI cases were diagnosed by toxin enzyme immunoassay (EIA), confirmed by toxigenic culture (n = 220) or toxin EIA alone (n = 188). Other cases were diagnosed by GDH detection and confirmed by toxin PCR (n = 101) or toxin EIA (n = 88), by toxin PCR alone (n = 91), toxin PCR and toxigenic culture (n = 72) or other diagnostic algorithms (n = 318).

The median age of patients was 72 years (IQR: 59–80); 38 (4%) CDI episodes were in those younger than 18 years, of whom 13 were younger than two years. The current hospital was reported as being the origin of infection for 66% (n = 673), another hospital for 18%

Table 2

Patient characteristics from 'light' (n = 1,078) and 'enhanced' surveillance[a] (n = 300) of *Clostridium difficile* infection in participating acute care hospitals in selected European countries[b], with putative determinants of a complicated course of infection and all-cause in-hospital mortality, 13 May–1 November 2013[c]

Patient characteristics	Light surveillance n[d]/N (%)	Enhanced surveillance n[d]/N (%)	Univariable analysis	
			Complicated course OR (95% CI)	In-hospital mortality OR (95% CI)
Age in years				
<65	370/1,077 (34)	104/299 (35)	ref.	ref.
65–84	549/1,077 (51)	152/299 (51)	3.4 (1.0–12.2)	1.6 (0.7–3.7)
≥85	158/1,077 (15)	43/299 (14)	6.6 (1.6–26.9)	2.1 (0.7–5.9)
Sex				
Female	573/1,078 (53)	157/300 (52)	ref.	ref.
Male	505/1,078 (47)	143/300 (48)	0.8 (0.3–1.8)	1.0 (0.5–2.1)
Recurrent infection				
No	862/978 (88)	240/277 (87)	ref.	ref.
Yes	116/978 (12)	37/277 (13)	0.7 (0.1–3.0)	0.9 (0.3–2.7)
CDI at admission				
No	505/984 (51)	153/276 (55)	ref.	ref.
Yes	479/984 (49)	123/276 (45)	1.7 (0.7–4.2)	0.3 (0.1–0.7)
Days of hospital stay to hospital-onset CDI				
Number (IQR)	11 (IQR: 6–21)	9 (IQR: 6–17)	NA	NA
CDI origin				
HA	885/1,078 (82)	249/300 (83)	ref.	ref.
CA	131/1,078 (12)	37/300 (12)	1.0 (0.3–3.7)	0.4 (0.1–1.6)
Unknown	62/1,078 (6)	14/300 (5)	2.0 (0.4–9.4)	1.1 (0.2–5.1)
Ward speciality				
Medical[e]	NC	194/299 (65)	ref.	ref.
Surgical	NC	53/299 (18)	0.9 (0.3–2.8)	0.8 (0.3–2.3)
ICU	NC	29/299 (10)	1.8 (0.6–5.8)	2.5 (1.0–6.5)
Other	NC	23/299 (8)	NA	0.7 (0.2–3.4)
Healthcare admission <3 months				
No	NC	84/287 (29)	ref.	ref.
Hospital	NC	194/287 (68)	1.0 (0.4–2.5)	1.3 (0.6–2.9)
Other	NC	9/287 (3)	1.6 (0.2–14.5)	1.0 (0.1–9.3)
Antibiotic treatment <3 months[f]				
No	NC	34/254 (13)	ref.	ref.
One course	NC	111/254 (44)	1.4 (0.4–5.2)	1.3 (0.4–4.1)
Multiple courses	NC	109/254 (43)	0.7 (0.2–3.0)	1.0 (0.3–3.3)
Expected survival in years (McCabe score)				
>5	NC	171/285 (60)	ref.	ref.
1–4	NC	83/285 (29)	2.2 (0.9–5.5)	1.8 (0.7–4.5)
<1	NC	31/285 (11)	2.5 (0.7–8.7)	12.0 (4.7–30.5)
Severe comorbidity (APACHE II CHP)[g]				
Liver cirrhosis	NC	16/295 (5)	0.7 (0.1–5.8)	1.7 (0.5–6.1)
NYHA class IV heart failure	NC	29/295 (10)	2.2 (0.7–7.0)	3.4 (1.4–8.3)
Pulmonary disease	NC	38/297 (13)	3.3 (1.2–8.5)	1.7 (0.7–4.3)
Chronic dialysis	NC	18/299 (6)	1.4 (0.3–6.7)	2.2 (0.7–7.2)
Immunocompromised status	NC	92/291 (32)	0.8 (0.3–2.2)	1.3 (0.6–2.7)
***C. difficile* clade**				
Clade 1, 3, 4 and 5	NC	187/267 (70)	ref.	ref.
Clade 2 (ribotype 027/176)	NC	80/267 (30)	0.9 (0.4–2.5)	1.0 (0.4–2.3)
***C. difficile* binary toxin genes**				
No	NC	165/264 (63)	ref.	ref.
Yes	NC	99/264 (38)	0.8 (0.3–2.1)	1.0 (0.4–2.1)

APACHE II CHP: Acute Physiology and Chronic Health Evaluation II chronic health points; CA: community-associated; CDI: *Clostridium difficile* infection; HA: healthcare-associated; ICU: intensive-care unit; IQR: interquartile range; NA: not applicable; NC: not collected; NYHA: New York Heart Association; OR: odds ratio; ref.: reference group.

[a] The 'light' surveillance option included patient data for CDI cases; in the 'enhanced' option, microbiological data on the first 10 CDI episodes per hospital were included.

[b] All 37 hospitals in 14 European countries (Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, the Netherlands, Norway, Poland, Romania, Serbia and United Kingdom (Scotland only)) tested the light option; 32 hospitals in 13 countries (Austria, Belgium, Denmark, Finland, France, Germany, Hungary, the Netherlands, Norway, Poland, Romania, Serbia and United Kingdom (Scotland only)) tested the enhanced option.

[c] Three-month assessment during this time period.

[d] Number of episodes/total number of episodes for which data were available, unless otherwise indicated.

[e] 'Medical' included several subspecialties of internal medicine (see protocol [14]).

[f] Antibiotic treatment in past 3 months was the only variable with >10% missing data.

[g] The reference group consisted of patients without the comorbidity listed.

Table 3

Surveillance indicators used to evaluate the ability to collect data and workload for the three surveillance options[a] for *Clostridium difficile* infection in 37 acute care hospitals in 14 European countries[b], 13 May–1 November 2013[c]

Country (number of hospitals in light/ enhanced surveillance)	Surveillance option								
	Minimal			Light			Enhanced		
	Testing frequency	Proportion of positive tests	Workload	Patient data available[f]	Data on CDI origin	Workload	Data on CDI outcome[g]	Matching PCR ribotype[h]	Workload
	Median number of tests per 10,000 patient days[d] (range)	n/N (%)	Median number of person-days per 10,000 hospital discharges[e] (range)	n/N (%)	n/N (%)	Median number of person-days per 10,000 hospital discharges[e] (range)	n/N (%)	n/N (%)	Median number of person-days per 10,000 hospital discharges[e] (range)
Austria (4/4)	31 (21–66)	111/1,117 (10)	0.7 (0.1–2.1)	111/117 (95)	109/111 (98)	2.1 (0.5–10.3)	40/40 (100)	26/34 (76)	2.8 (1.0–3.0)
Belgium (3/3)	55 (50–85)	60/833 (7)	0.3 (0.1–0.8)	53/53 (100)	52/53 (98)	1.6 (1.5–2.2)	26/28 (93)	16/26 (62)	1.6 (0.8–4.4)
Denmark (4/4)	71 (43–105)	202/1,360 (15)	0.5 (0.3–0.9)	168/171 (98)	163/168 (97)	1.0 (0.9–2.0)	37/39 (95)	NA	1.7 (1.3–2.5)
Estonia (2/0)[i]	17 (10–24)	17/218 (8)	NA	17/18 (94)	17/17 (100)	NA	NA	NA	NA
Finland (3/1)	129 (33–151)	48/448 (11)	1.2 (0.8–4.2)	23/29 (79)	23/23 (100)	3.3 (1.2–4.2)	10/10 (100)	9/10 (90)	5.0[j]
France (2/1)	72 (63–81)	35/493 (7)	NA	40/46 (87)	39/40 (98)	NA	10/10 (100)	5/9 (56)	NA
Germany (3/3)	82 (70–111)	174/2,656 (7)	1.0 (0.1–1.8)	171/174 (98)	153/171 (89)	1.2 (0.5–1.8)	30/30 (100)	21/27 (78)	2.1 (1.2–3.0)
Hungary (2/2)	77 (67–86)	237/1,192 (20)	2.5 (2.0–3.0)	251/254 (99)	236/251 (94)	38.7 (28.4–49.0)	19/20 (95)	14/17 (82)	9.6 (4.1–15.1)
Netherlands (3/3)	45 (7–262)	79/1,124 (7)	1.7 (0.6–1.8)	43/43 (100)	38/43 (88)	1.8 (1.7–5.1)	29/29 (100)	NA	5.3 (4.0–13.6)
Norway (2/2)[i]	38 (23–52)	60/614 (10)	0.8[j]	60/60 (100)	55/60 (92)	1.5[j]	20/20 (100)	12/18 (67)	2.3[j]
Poland (2/2)[i]	20 (18–21)	79/173 (46)	NA	34/69 (49)	34/34 (100)	NA	19/19 (100)	16/16 (100)	NA
Romania (2/2)[i]	308 (9–607)	26/427 (6)	NA	26/33 (79)	24/26 (92)	NA	12/13 (92)	NA	NA
Serbia (3/3)[i]	40 (7–184)	49/253 (19)	15.0 (2.9–26.4)	49/49 (100)	49/49 (100)	15.0 (2.9–26.4)	30/30 (100)	NA	37.4 (5.9–92.2)
UK-Scotland (2/2)	179 (142–216)	33/1,813 (2)	2.2 (2.1–2.3)	32/36 (89)	24/32 (75)	4.7 (2.1–7.3)	12/12 (100)	9/9 (100)	3.7 (1.2–6.3)
Total (37/32)	**58 (7–607)**	**1,210/12,721 (10)**	**1.1 (0.1–26.4)**	**1,078/1,152 (94)**	**1,016/1,078 (94)**	**2.0 (0.5–49.0)**	**294/300 (98)**	**128/166 (77)**	**3.0 (0.8–92.2)**

CDI: *Clostridium difficile* infection; NA: not available; UK-Scotland: United Kingdom (Scotland only).

The pilot study was based on a non-representative sample, thus the results presented in this table cannot be interpreted as being representative of any participating country or of the European Union/European Economic Area.

Missing values indicate that hospitals did not participate in enhanced surveillance and/or did not reply to the feasibility questionnaire.

[a] Three surveillance options were tested: 'minimal' (aggregated hospital data), 'light' (including patient data for CDI cases) and 'enhanced' (including microbiological data on the first 10 CDI episodes per hospital).

[b] Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, the Netherlands, Norway, Poland, Romania, Serbia, United Kingdom (Scotland only) carried out minimal and light surveillance. Austria, Belgium, Denmark, Finland, France, Germany, Hungary, the Netherlands, Norway, Poland, Romania, Serbia and United Kingdom (Scotland only) also carried out enhanced surveillance.

[c] Three-month assessment during this time period.

[d] Median testing of the country's participating hospitals.

[e] Workload needed to complete the surveillance option, as reported by 26 respondents who completed the feasibility questionnaire.

[f] Number of patients with clinical data available, divided by the number of patients reported by minimal surveillance, expressed as a percentage.

[g] Percentage of patients for whom the presence or absence of a complicated in-hospital outcome (as defined in the Box) was identified.

[h] Percentage of isolates of which the reported ribotype matched the results of the coordinating laboratory.

[i] Countries without an implemented national surveillance of CDI at the start of the European *Clostridium difficile* Infection Surveillance Network (ECDIS-Net) project.

[j] One hospital provided a response to this question, therefore no range was calculable.

(n = 178), a long-term care facility for 1% (n = 13) and another healthcare facility for 2% (n = 21) of the 1,016 CDI episodes of known origin (for 62 episodes, the origin was unknown). Other patient characteristics are shown in Table 2.

Enhanced surveillance: complicated CDI and in-hospital mortality

For 300 CDI episodes in 32 hospitals, enhanced surveillance data were also submitted (Table 2). The course of CDI was known for 98% (n = 294) of cases; 8% (n = 24) experienced a complicated course of infection (as defined in the Box). In univariable analysis, a complicated course was associated with age of 85 years or older and severe pulmonary disease, but not with CDI origin, presence of PCR ribotypes 027 or 176, or of binary toxin genes (Table 2). A total of 12% (n = 37) of CDI cases died during hospitalisation. Six deaths (2% of all CDI episodes) were related to CDI, 23 deaths (8% of all CDI episodes) were unrelated to CDI, and the relationship between CDI and death was unknown for the remaining eight episodes (3% of all CDI episodes). Patients with a complicated course had a 42% risk of in-hospital death (of which 25% were CDI-related) compared with 9% among patients with an uncomplicated course. All-cause in-hospital mortality was associated with a lower number of years of expected survival (a high McCabe score), healthcare-onset CDI and severe heart failure, but not with CDI origin, presence of PCR ribotypes 027 or 176, or of binary toxin genes (Table 2).

Enhanced surveillance: microbiological data

C. difficile was cultured and characterised in the coordinating laboratory for 267 (89%) of the 300 CDI episodes registered during enhanced surveillance. The presence of toxin A and B genes was confirmed in 99% (263/265) of the cultured isolates; binary toxin genes were present in 38% (99/264) of the isolates. A total of 51 different PCR ribotypes were characterised. The predominant PCR ribotype was 027 (30%; n = 79), followed by the highly related PCR ribotypes 014 and 020 (15%; n = 40), and PCR ribotype 001 (6%; n = 15). PCR ribotype 027 was identified in isolates from eight European countries in 4–85% of all characterised samples, depending on the country (Figure 2).

PCR ribotype 176, which is highly related to 027, was found in one CDI case in a country where no PCR ribotype 027 isolates were identified. The proportion of PCR ribotype 027 isolates correlated with the incidence rate of HA-CDI per 10,000 patient-days (Spearman's rho: 0.64; 95% CI: 0.36–0.81) (Figure 3).

All isolates that were investigated for antimicrobial susceptibility (n = 251) were susceptible in vitro to metronidazole. Eight PCR ribotype 027 isolates from Austria, Germany and Hungary showed reduced susceptibility to metronidazole, with a MIC just below the EUCAST epidemiological cut-off value [24]. Two PCR ribotype 027 isolates from Denmark showed reduced susceptibility to vancomycin, with a MIC just below the EUCAST epidemiological cut-off value [24]; however, resistance to vancomycin was not detected. In vitro moxifloxacin resistance was identified in 37% (n = 92) isolates, of which 77% (n = 71) belonged to PCR ribotype 027.

Feasibility and workload

Participating hospitals reported a median of seven CDI episodes (IQR: 4–12) per month through both minimal and light surveillance. The feasibility questionnaire was completed by 26 of the 37 participating hospitals. Completion of the light and enhanced options were found to be 'not difficult' for 23/26 and 21/24 respondents, respectively. The remaining respondents found them 'quite difficult'.

The median workload for the 'minimal', 'light' and 'enhanced' surveillance options was 1.1, 2.0 and 3.0 person-days per 10,000 hospital discharges, respectively (Table 3).

The highest workload was reported by countries with the highest aggregated CDI incidence rates during the pilot (Serbia and Hungary). There were no differences in surveillance indicators by pre-existing surveillance activities, or when considering laboratory or typing capacity for CDI in the pilot study (Table 3).

Data quality

Completeness of data was 94% (1,078/1,152) for patient data in the light option and 98% (294/300) for data on the course of CDI in the enhanced option. Testing frequency (range: 17–308 tests per 10,000 patient days) and the proportion of positive tests (range: 2–46%) varied between countries (Table 3). The testing frequency correlated with the overall CDI incidence rate per 10,000 patient days (Spearman's rho: 0.45; 95% CI: 0.15–0.68). PCR ribotyping results from the NRLs obtained during enhanced surveillance were concordant with the coordinating laboratory's results for 77% (128/166) of the isolates. Discordant results were either due to a mismatch in the identified PCR ribotype (n = 19; 11%), or because a PCR ribotype pattern result was not recognised by a NRL (n = 17; 10%) or by the coordinating laboratory (n = 2; 1%). External quality assessment demonstrated 75% and 86% accuracy of PCR ribotype allocation by the NRLs in 2013 and 2014, respectively.

Discussion

CDIs are a major concern for hospitals in Europe. The first ECDC point prevalence survey in 2011–12 estimated that 123,997 patients (95% CI: 107,697–441,969) developed a HA-CDI within the European Union each year [9]. In the United States, CDI has been declared an 'urgent threat' [25], with an estimated 80,400 HA-CDI cases in 2011 [26]. Establishing Europe-wide surveillance of CDIs is a pre-requisite to controlling these infections in Europe. In 2011, 14 European countries had national CDI surveillance, but methodologies varied, and only four countries regularly linked *C. difficile* microbiological results to epidemiological data [3]. Therefore, a standardised protocol was proposed for periodical

or continuous CDI surveillance in European acute care hospitals, allowing direct interhospital and intercountry comparison of surveillance results.

Feasibility

Results of our study in which we piloted a standardised surveillance protocol for CDI for European acute care hospitals suggests that all three surveillance options were manageable in participating countries, regardless of the countries' pre-established level of CDI surveillance and microbiological typing capacity. Completeness of data was high, and hospital participants reported that the workload was manageable. Nevertheless, modifications were made on the surveillance methodology and forms to further optimise data collection. The finalised protocol version 2.2 is now available on the website of ECDC [27].

Epidemiological and microbiological findings

Using the pilot protocol, participating hospitals could obtain detailed information on the local epidemiology of CDI at their respective facilities that could be used to target and reinforce infection prevention and control measures and resources. This pilot study had an important impact on certain national CDI-related activities as well: three of five participating countries that did not have national CDI surveillance at start of the ECDIS-Net project reported a high percentage of PCR ribotype 027 isolates in this study, and two of these countries (Poland [28] and Romania) decided to continue with intensified CDI surveillance. Interest in the surveillance and completeness of results also suggests that wide-scale implementation at national and European level would be successful in acute care hospitals.

Although the non-representative selection of hospitals does not allow for interhospital or intercountry comparisons in the pilot study, patients enrolled in the enhanced option permitted a more in-depth analysis of the pilot data collected, allowing us to assess the relationship between patient and microbiological characteristics and in-hospital outcome of CDI, our secondary objective. Similar to the findings of a European study performed in 2008 [2], the majority of the patients in our pilot study had risk factors for CDI (e.g. median age of 72 years and 87% had used antibiotics in the previous three months). We found plausible associations between certain comorbidity variables and a complicated course of CDI or all-cause in-hospital mortality of CDI cases; however, the presence of PCR ribotypes 027 and 176 was not associated with a higher risk of all-cause in-hospital death, as found in a larger study in the United Kingdom in 2006–11 [29]. In contrast, the proportion of PCR ribotypes 027 isolates correlated with a higher incidence rate of HA-CDI, thus corroborating existing evidence on the high potential of this *C. difficile* PCR ribotype to spread. Indeed, this fluoroquinolone-resistant strain that emerged in Europe in 2004 [13] was the most frequently isolated ribotype, particularly in participating hospitals of eastern European countries. This finding is in line with the 'European, multicentre, prospective, biannual, point-prevalence study of *C. difficile* infection in patients admitted with diarrhoea' (EUCLID) study (2011–13) that found PCR ribotype 027 to be most prevalent, clustering in Germany, Hungary, Poland and Romania [12].

Resistance to antibiotics that are routinely used to treat CDIs such as metronidazole and vancomycin was not detected in our study. Two PCR ribotype 027 isolates from one hospital showed a decreased susceptibility to vancomycin (MIC = 2 mg/L), but the clinical relevance of this finding is uncertain.

Data quality

We found varying frequencies of testing for CDI and percentages of positive tests in participating hospitals and countries, primarily indicating the need for an update of the European diagnostic guideline [30] and for promotion of optimal ascertainment of CDI. In addition, there is a need to address local or national variations in CDI case finding, ascertainment and reporting, which may be substantial across Europe, due to probable differences in clinical and laboratory awareness, practices of specimen collection from diarrhoeic patients and specimen transport, clinical and laboratory indications, requests from physicians and CDI testing methods, local epidemiology (e.g. intensified testing during outbreaks), financial resources to test for CDI, data sources for surveillance, and reporting incentives or disincentives. Therefore, we suggest that in CDI surveillance programmes the possibility of adjusting CDI incidence rates at least for key factors related to sampling and testing methods should be investigated. We recommend that validation studies accompany national surveillance to estimate sensitivity and specificity, in order to correct national and European CDI infection rate estimates.

Furthermore, standardisation of PCR ribotyping is essential for implementation of the enhanced surveillance option, as results show suboptimal concordance between results of national and external laboratories. Agarose-based ribotyping results are more difficult to interpret and to exchange between laboratories than capillary-based results [31]. The increase, from 23% in 2011 to 50% in 2014, in the percentage of ECDIS-Net participating countries that use capillary-based PCR ribotyping [18] was the most likely explanation for the better performance in the external quality control exercise in 2014 [31]. Further standardisation of PCR ribotyping will likely be achieved by regular exchange of new *C. difficile* strains and build-up of a consistent reference database. The first steps have already been taken by concerted action of ECDIS-Net members with reference laboratories from CDC and the Public Health Agency of Canada [31]. At the same time, new developments in DNA sequence analyses should be monitored closely for application in ribotyping modifications and considered for implementation in surveillance activities of *C. difficile* [32]. In our pilot study, PCR ribotyping of the first 10 strains per hospital in the enhanced

option was performed to balance effort, costs and benefits, such as in the national surveillance programme of Belgium [5]. Despite these positive experiences, further evidence for this approach should be obtained and evaluated at European level.

Other limitations

The results of our pilot study are not generalisable to all European acute care hospitals as it was based on a non-representative convenience sample, as also indicated by the disproportionally high number of tertiary care hospitals (21/37) in our sample. Similarly, our analytical epidemiological results and country-specific results are based on very small numbers of hospitals and should not be considered as representative. Specifically, the number of events allowed for univariable analysis only when exploring associations between covariables and outcome of CDI. Assessing the local context in more details (e.g. gathering information on clinical practices and/or policies related to specimen collection and CDI testing in the participating hospitals) or covering all CDC surveillance evaluation attributes [33] was beyond the scope of this pilot study. Local audits to determine surveillance sensitivity, in both case finding and collection of denominator data, could have helped to elucidate some of the larger observed variations.

Conclusions

We conclude that continuous or periodical surveillance with collection of different levels of epidemiological and microbiological data following a standardised protocol is a feasible strategy to monitor CDIs in European acute care hospitals. Ideally, national and international validation studies, regular and comprehensive evaluation of the surveillance protocol, as well as CDI case finding, ascertainment and reporting should complement the surveillance activity.

ECDC has used the final protocol version 2.2 to initiate CDI surveillance in EU/EEA countries in 2016, and will gradually incorporate enhanced surveillance data in The European Surveillance System (TESSy) [27,34]. Importantly, the surveillance of CDI in European acute care hospitals will be the first Europe-wide, hospital-based surveillance of a primarily healthcare-associated infection with a distinct microbiological component. The protocol can be used as a tool to guide local CDI surveillance and ultimately contribute to reducing CDI incidence rates in acute care hospitals. Finally, aggregated data from nationally representative samples should allow an estimation of the true incidence rate of CDIs in Europe.

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Conflict of interest

Sofie M van Dorp, Pete Kinross, Petra Gastmeier, Michael Behnke, Axel Kola, Michel Delmée, Anastasia Pavelkovich, Agnes Hajdu, André Ingebretsen, Hanna Pituch, Milica Jovanović, Camilla Wiuff, Daniela Schmid, Katharina EP Olsen, Carl Suetens: none declared.

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Authors' contributions

PG and EJK led the project. CS developed the surveillance protocol in collaboration with all co-authors and analysed data. SD coordinated the study, analysed the data and drafted the manuscript with PK, AH, and EJK. All co-authors contributed to data collection and reviewed the manuscript.

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Diversity of *Clostridium difficile* PCR ribotypes in Europe: results from the European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea (EUCLID), 2012 and 2013

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Clostridium difficile **infection (CDI) is the major cause of infective diarrhoea in healthcare environments. As part of the European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea (EUCLID), the largest *C. difficile* epidemiological study of its type, PCR ribotype distribution of *C. difficile* isolates in Europe was investigated. PCR ribotyping was performed on 1,196 *C. difficile* isolates from diarrhoeal samples sent to the European coordinating laboratory in 2012–13 and 2013 (from two sampling days) by 482 participating hospitals from 19 European countries. A total of 125 ribotypes were identified, of which ribotypes 027 (19%, n =222), 001/072 (11%, n = 134) and 014/020 (10%, n = 119) were the most prevalent. Distinct regional patterns of ribotype distribution were noted. Of 596 isolates from patients with toxin-positive stools (CDI cases), ribotype 027 accounted for 22% (32/144) of infections in cases aged from 18 to less than 65 years, but the prevalence decreased in those aged ≥ 65 years (14% (59/412)) and further decreased in those aged ≥ 81 years (9% (18/195)). The prevalence of ribotype 027 and 176, but not other epidemic strains, was inversely proportional to overall ribotype diversity ($R^2 = 0.717$). This study highlights an increased diversity of *C. difficile* ribotypes across Europe compared with previous studies, with considerable intercountry variation in ribotype distribution. Continuous surveillance programmes are necessary to monitor the changing epidemiology of *C. difficile*.**

Introduction

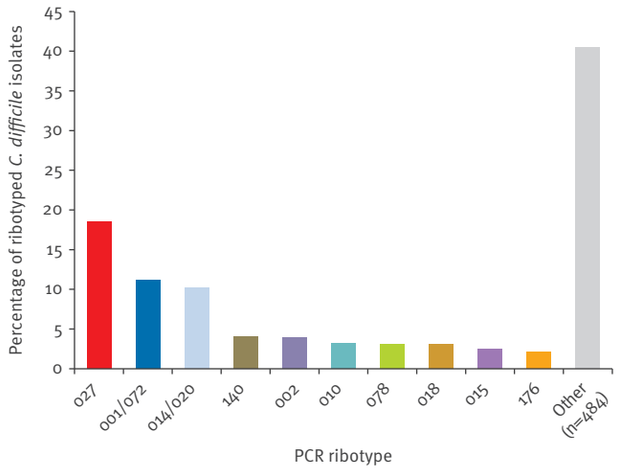
Clostridium difficile is the most common cause of infective diarrhoea in hospitalised patients, and is associated with substantial morbidity and mortality. Over the past decade, the burden of *C. difficile* infection (CDI) has increased in many European countries, with the annual incidence in Europe estimated at 124,000 cases in 2011–12 [1] with all-cause mortality rates of 3–30% [2-5]. CDI continues to be the focus of comprehensive national-level control and surveillance programmes in some countries, but the public health threat of CDI is not yet fully recognised across Europe.

C. difficile is an intensively typed pathogen, with a wide range of methods applied to understand its epidemiology. The emergence of so-called 'hypervirulent' *C. difficile* types has intensified the challenge of CDI. In the 1990s, strains belonging to PCR ribotype 027 (also referred to as restriction endonuclease type BI and North American pulsed-field type 1 (NAP-1)) were infrequently isolated from patients with CDI [6] but in the last decade this type has become highly represented among clinical isolates across Europe [7], with ribotype 027 often linked to outbreaks with increased disease severity [8-10]. In a 2008 study of *C. difficile* epidemiology in Europe, which consisted of a network of 106 laboratories in 34 countries, 65 different ribotypes were identified, of which ribotypes 014/020 (16%), 001 (9%) and 078 (8%) were the most prevalent [11]. Ribotype 027 accounted for 5% of all *C. difficile* isolates.

The European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea (EUCLID) is the largest and most recent epidemiological study of *C. difficile*, encompassing 482 participating hospitals from 20 European countries (Austria, Belgium, Bulgaria,

Figure 1

Distribution of the 10 most commonly isolated *Clostridium difficile* PCR ribotypes from all *C. difficile* isolates in the participating countries, EUCLID, 2012–13 and 2013[a] (n = 1,196)



EUCLID: European, multicentre, prospective, biannual, point-prevalence study of Clostridium difficile infection in hospitalised patients with diarrhoea.

The percentages are the based on the total number of ribotyped C. difficile isolates.

Data from the following countries (n= 19) were included: Austria, Belgium, Bulgaria, Czech Republic, Finland, France, Germany, Greece, Hungary, Ireland, Italy, the Netherlands, Poland, Portugal, Romania, Slovakia, Spain, Sweden and United Kingdom. None of the faecal samples submitted from Slovenia during the two sampling days were found to be positive for C. difficile or its toxins.

[a] The countries submitted inpatient diarrhoeal samples on two sampling days (one day in winter, in December 2012 or January 2013, and one day in summer, in July or August 2013.

Czech Republic, Finland, France, Germany, Greece, Hungary, Ireland, Italy, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden and United Kingdom (UK)) [12]. The study measured the prevalence and underdiagnosis of CDI on two sampling days (one in winter and one in summer) in 2012 and 2013; participating hospitals forwarded inpatient diarrhoeal faecal samples to national coordinating laboratories for CDI testing by a study reference method. The mean measured rate of CDI was 7.0 cases (country range 0.7–28.7) per 10,000 patient-bed days and, across all hospitals on the two sampling days, 148/641 (23%) samples positive for CDI were not diagnosed by participating hospitals due to lack of clinical suspicion; a further 68 samples were not diagnosed due to sub-optimal laboratory diagnostic methods [12].

Here, we report the PCR ribotype distribution of *C. difficile* isolates in Europe from the 1,211 samples, including those from 595 patients with confirmed CDI, that were culture positive for *C. difficile* in EUCLID and discuss the changing epidemiology of CDI from previous ribotype surveillance studies.

Methods

Study design

EUCLID followed the design of a previous point-prevalence study in Spain [13] and full methodology can be found in a previous EUCLID publication [12]. Briefly, the study was coordinated from the European coordinating laboratory in Leeds, UK. A national coordinating laboratory was selected for each of the 20 participating European countries and the national coordinators selected hospitals to cover all major geographical regions within each country. Hospitals were recruited at a rate of one per million population in all countries. All inpatient diarrhoeal samples submitted to the microbiology laboratory of the participating hospital on two sampling days (one day in winter, in December 2012 or January 2013, and one day in summer, in July or August 2013) were eligible for inclusion. Anonymised samples were sent from the participating hospital, within seven days, to the national coordinating laboratory for their country, where they were tested for CDI and cultured for *C. difficile*. Transport was refrigerated for six countries (Finland, France, Hungary, Portugal, Romania and Spain) in the winter sampling period and for all 20 countries in the summer.

Patients were defined as a CDI case if their faecal sample was positive according to a two-stage algorithm: membrane enzyme immunoassay for glutamate dehydrogenase and *C. difficile* toxins A and B (C DIFF QUIK CHEK COMPLETE, Techlab, United States). The incidence of CDI in children aged under 2 years, in whom diarrhoeal illness is common and *C. difficile* carriage rates are high [14], is unclear [15,16]. Patients under 2 years-old who tested positive for free toxin in the stool were therefore not included as cases of CDI.

C. difficile colonisation of patients was assumed for those whose faecal sample was positive for culture of *C. difficile* but negative for free *C. difficile* toxin.

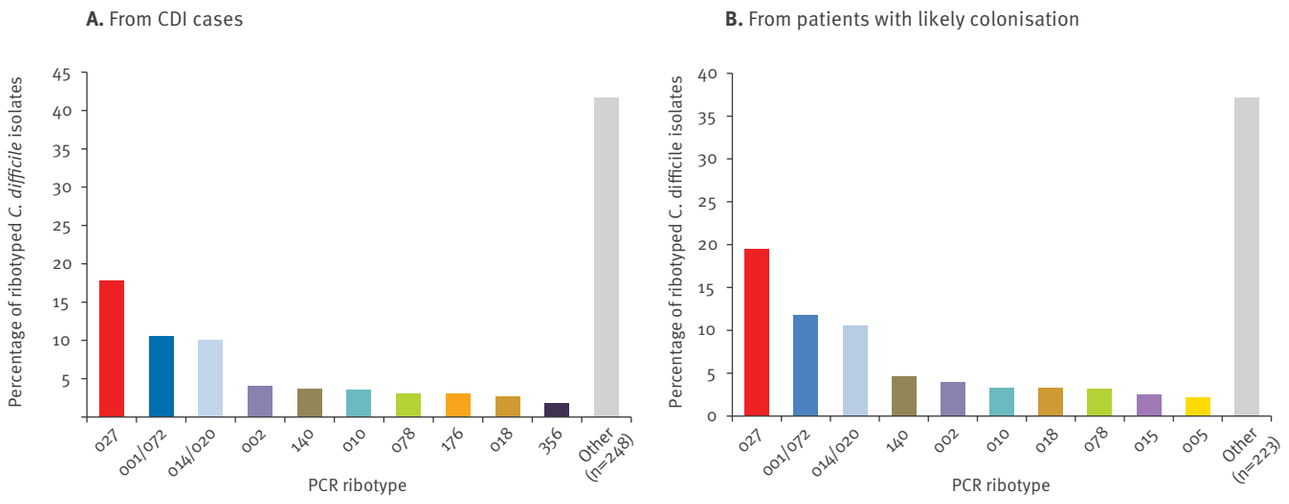
PCR ribotyping analysis

Isolates of *C. difficile* were stored in brain-heart infusion broth supplemented with 10% glycerol at the national coordinating laboratories, before being frozen and transported to the European coordinating laboratory in Leeds, UK. All *C. difficile* isolates identified at national coordinating laboratories (regardless of whether or not the samples were positive for glutamate dehydrogenase and free toxin, indicating CDI) were sent to Leeds, to confirm pathogen identification and for PCR ribotyping analysis. PCR ribotyping was performed on all *C. difficile* isolates using the previously published capillary gel-based method [17].

Geographical distribution of ribotypes was based on the United Nations geoscheme for Europe [18]: Northern Europe (Finland, Ireland, Sweden and UK),

Figure 2

Distribution of the 10 most commonly isolated *Clostridium difficile* PCR ribotypes in isolates from (A) cases of *C. difficile* infection[a] (596 isolates) and (B) patients with likely colonisation[b] (600 isolates), EUCLID, 2012–13 and 2013[c]



CDI: *Clostridium difficile* infection; EUCLID: European, multicentre, prospective, biannual, point-prevalence study of Clostridium difficile infection in hospitalised patients with diarrhoea.

The percentages shown are the based on the total number of ribotyped *C. difficile* isolates.

Data from the following countries (n = 19) were included: Austria, Belgium, Bulgaria, Czech Republic, Finland, France, Germany, Greece, Hungary, Ireland, Italy, the Netherlands, Poland, Portugal, Romania, Slovakia, Spain, Sweden and United Kingdom. None of the faecal samples submitted from Slovenia during the two sampling days were found to be positive for *C. difficile* or its toxins.

[a] Positive for free *C. difficile* toxin, tested using a two-stage algorithm (membrane enzyme immunoassay for glutamate dehydrogenase and C. difficile toxins A and B).

[b] Positive for culture of *C. difficile* but negative for free C. difficile toxin.

[c] The countries submitted inpatient diarrhoeal samples on two sampling days (one day in winter, in December 2012 or January 2013, and one day in summer, in July or August 2013).

Western Europe (Austria, Belgium, France, Germany and the Netherlands), Southern Europe (Greece, Italy, Portugal and Spain) and Eastern Europe (Bulgaria, Czech Republic, Hungary, Poland, Romania and Slovakia). None of the faecal samples submitted from Slovenia during the two sampling days were found to be positive for *C. difficile* or its toxins.

Statistical analyses

Simpson's index (D) was used to compare diversity in ribotype distribution among countries and patient age groups, and was calculated as follows: $D = \Sigma(n(n-1))/N(N-1)$, where n represents the total number of isolates of a particular ribotype and N represents the total combined number of isolates for all ribotypes. For ease of illustration, Simpson's reciprocal index (1/D) was plotted, where the lowest possible diversity is 1 (a population dominated by a single ribotype) and increasing values indicate increasing diversity.

Chi-squared test was used to compare the proportion of ribotypes from CDI cases among patient age groups.

Results

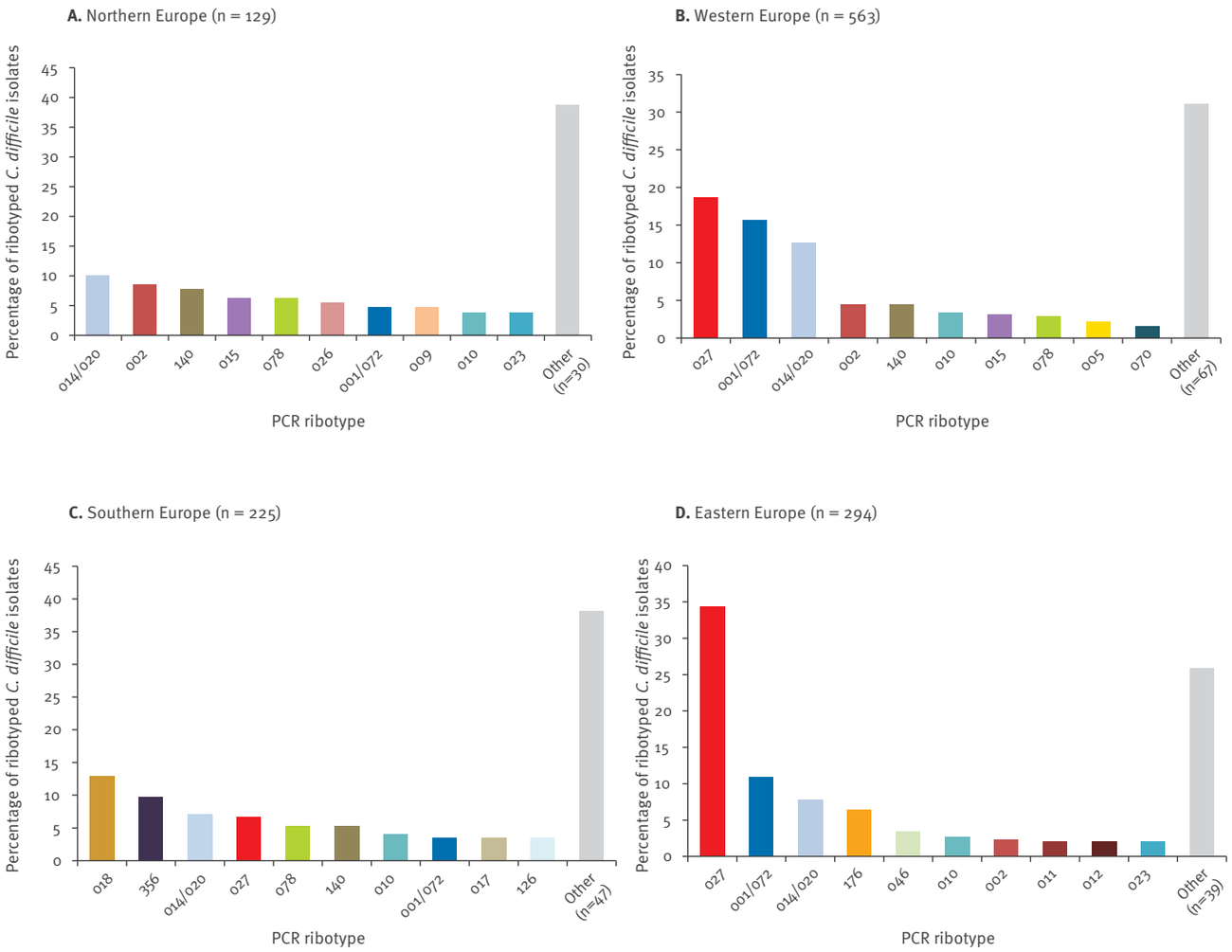
Samples obtained for PCR ribotyping analysis

A total of 3,923 and 3,389 faecal samples were submitted during the winter and summer testing periods, respectively. A total of 15 samples were excluded due to incomplete data, giving a total of 7,297 samples for analysis.

A PCR ribotype was assigned to 1,194 of the 1,211 *C. difficile* isolates received by the European coordinating laboratory after removal of 17 sporadic isolates that could not be assigned to a ribotype (obtained from 19 countries). For two samples more than one ribotype was isolated, giving a total of 1,196 *C. difficile* isolates. The median age of patients for whom a *C. difficile* PCR ribotype was reported was 71 years (range: 1–99) and patient ward locations included medical (n = 704), intensive therapy unit/high dependency unit (n = 47), obstetrics and gynaecology (n = 4), paediatric (n = 138) and surgery (n = 106).

Figure 3

PCR ribotype diversity of *Clostridium difficile* isolates by European region[a], EUCLID, 2012–13 and 2013[b] (n = 1,196)



EUCLID: European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea.

The charts show the proportion of the most common ribotypes per region; the percentages are the based the number of typed isolates in the region.

[a] The 19 participating countries were classified by European region as defined according to the United Nations geoscheme for Europe [18]: (A) Northern Europe: Finland, Ireland, Sweden and United Kingdom; (B) Western Europe: Austria, Belgium, France, Germany and the Netherlands; (C) Southern Europe: Greece, Italy, Portugal and Spain; and (D) Eastern Europe: Bulgaria, Czech Republic, Hungary, Poland, Romania and Slovakia. None of the faecal samples submitted from Slovenia during the two sampling days were found to be positive for *C. difficile* or its toxins.

[b] The countries submitted inpatient diarrhoeal samples on two sampling days (one day in winter, in December 2012 or January 2013, and one day in summer, in July or August 2013).

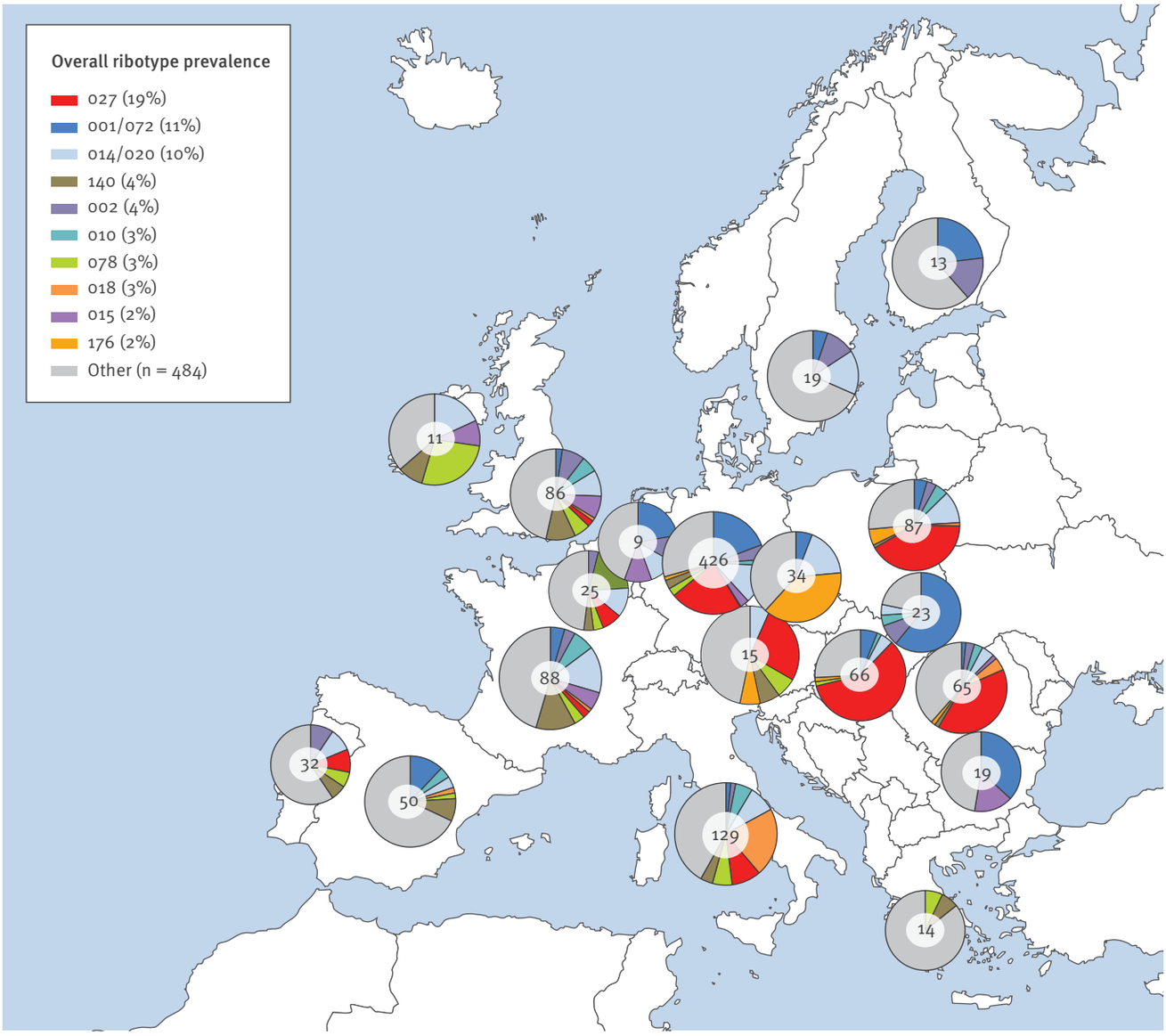
C. difficile PCR ribotype diversity in Europe

We identified *C. difficile* isolates belonging to 125 different ribotypes across 19 countries; the 10 most commonly isolated ribotypes received by the European coordinating laboratory are shown in Figure 1. *C. difficile* ribotype 027 was the most prevalent (19%, n =222); ribotypes 001/072 (11%, n = 134) and 014/020 (10%, n = 119) were the second and third most prevalent, respectively. Ribotype 078, the third most prevalent ribotype in a previous study in 2008 [11], accounted for 3% (n = 37) of isolates in our study.

Of the 1,196 *C. difficile* isolates where a PCR ribotype was identified, 596 were isolated from stool samples of 595 CDI cases (positive for free *C. difficile* toxin), while 600 were from 599 patients who were likely to be colonised (positive for culture of *C. difficile* but negative for free *C. difficile* toxin). The 10 most commonly

Figure 4

Geographical distribution of *Clostridium difficile* PCR ribotypes, by participating European country[a], EUCLID, 2012–13 and 2013[b] (n = 1,196)



EUCLID: European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea.

Pie charts show the proportion of the most common ribotypes per country and the number in the centre of the charts is the number of typed isolates in the country.

[a] Austria, Belgium, Bulgaria, Czech Republic, Finland, France, Germany, Greece, Hungary, Ireland, Italy, the Netherlands, Poland, Portugal, Romania, Slovakia, Spain, Sweden and United Kingdom. None of the faecal samples submitted from Slovenia during the two sampling days were found to be positive for *C. difficile* or its toxins.

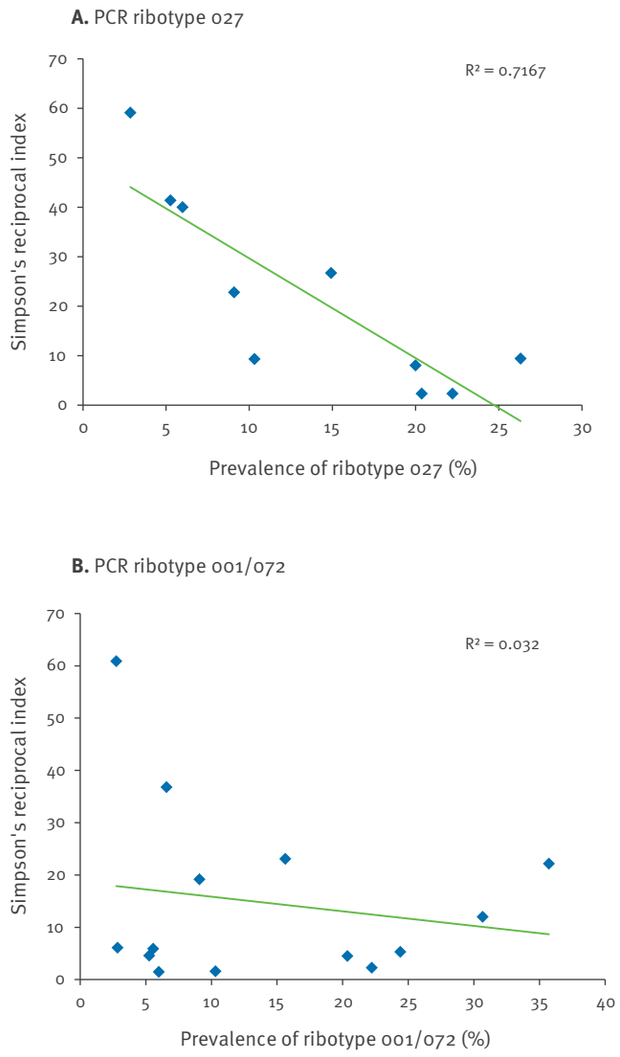
[b] The countries submitted inpatient diarrhoeal samples on two sampling days (one day in winter, in December 2012 or January 2013, and one day in summer, in July or August 2013).

isolated ribotypes from samples from CDI cases (Figure 2A) and those from patients with likely *C. difficile* colonisation (Figure 2B) were compared. The ribotype distribution was found to be largely similar between CDI cases and patients with likely colonisation, suggesting no obvious over-representation of *C. difficile* isolates associated with colonisation or infection.

The geographical distribution of all *C. difficile* ribotypes isolated in this study is summarised in Figures 3 and 4. Many of the most commonly isolated ribotypes were

Figure 5

Relationship between Simpson's reciprocal index of Clostridium difficile PCR ribotype diversity and EUCLID-measured prevalence[a] of (A) ribotype 027[b] (n = 222) and (B) ribotype 001/072[c] (n = 134), EUCLID, 2012–13 and 2013[d]



EUCLID: European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficil*e infection in hospitalised patients with diarrhoea.

[a] As reported in [12].

[b] The 10 countries where ribotype 027 was isolated were Austria, Belgium, France, Germany, Hungary, Italy, Poland, Portugal, Romania, United Kingdom.

[c] The 14 countries where ribotype 001/072 was isolated were Bulgaria, Czech Republic, Finland, France, Germany, Hungary, Italy, Netherlands, Poland, Romania, Slovakia, Spain, Sweden, United Kingdom.

[d] The countries submitted inpatient diarrhoeal samples on two sampling days (one day in winter, in December 2012 or January 2013, and one day in summer, in July or August 2013).

found across each region (Figure 3). Among these were ribotype 001/072 (in 14 countries), 014/020 (in 16 countries), 002 (in 12 countries), 078 (in 11 countries) and 027 (in 10 countries). However, we also observed substantial variation in ribotype distribution among the study countries (Figure 4). For example, ribotypes 018 and 356 were commonly isolated in Italy (22% (28/129) and 17% (22/129) prevalence, respectively) but were rarely isolated in other countries. Similarly, ribotype 176, which is closely related to ribotype 027, represented 38% (13/34) of all isolates in the Czech Republic, but only 2% (26/1,196) of total isolates. Distinct regional patterns in the distribution of *C. difficile* ribotypes were noted (Figure 4).

The proportion of samples that were positive and negative for free *C. difficile* toxin received from each participating country was similar, suggesting that the geographical distribution of ribotypes was not influenced by over- or under-representation of isolates associated with either CDI cases or likely colonisation.

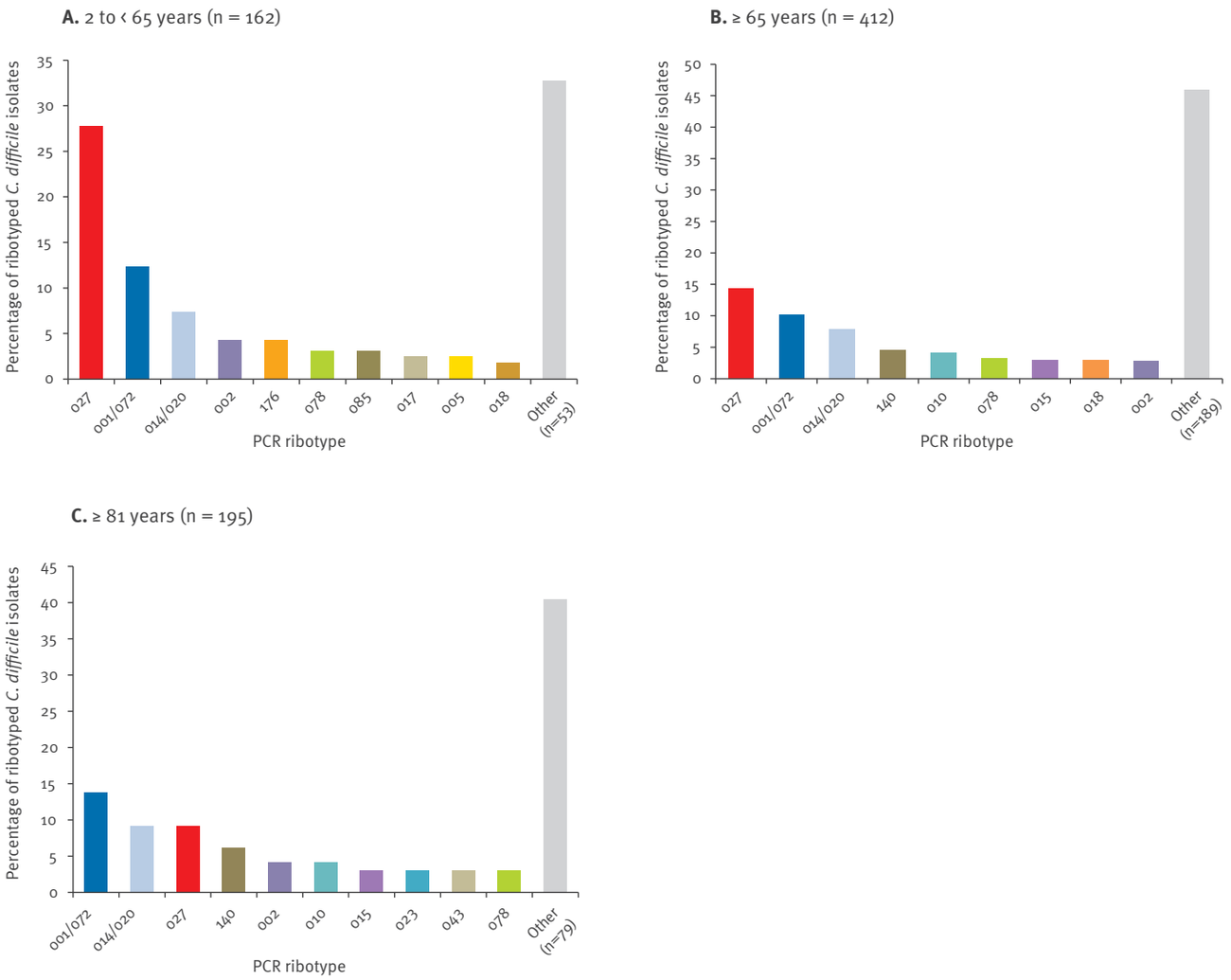
Relationship between ribotype diversity and prevalence of ribotype 027

Given that most ribotype 027 strains were found to be localised mainly to four countries (Germany, Hungary, Poland and Romania) [12], we investigated the relationship between prevalence of ribotype 027 in the 10 countries in which it was identified in EUCLID and the overall ribotype diversity among all *C. difficile* isolates (from CDI cases and those with likely colonisation) received during the study from those countries. Using Simpson's reciprocal index of diversity, we found that ribotype diversity decreased as the prevalence of ribotype 027 increased in the 10 countries where ribotype 027 was isolated ($R2 = 0.717$; Figure 5A). To determine whether this was a common feature of epidemic *C. difficile* types, we performed the same analysis on ribotype 001/072 from the 14 countries where this type was isolated, but noted no obvious linear relationship between country ribotype diversity and prevalence of 001/072 ($R2 = 0.032$; Figure 5B). Taken together, these data suggest that countries with a high prevalence of ribotype 027 strains have a lower overall ribotype diversity than countries with a low prevalence of ribotype 027.

A similar level of endemicity was observed in EUCLID for ribotype 176 in the Czech Republic (38% (13/34) of all ribotypes in the Czech Republic). *C. difficile* ribotype 176 is thought to share many similarities to ribotype 027 [19] and it has been suggested that this type may often be misdiagnosed as a ribotype 027 infection [20]. Therefore, we repeated this analysis to include both ribotype 027 and ribotype 176 and the findings were similar, with ribotype diversity decreasing as the prevalence of ribotypes 027 and 176 increased ($R2 = 0.722$; data not shown). This suggests that our observation is not limited to ribotype 027 and may extend to other closely related ribotypes with epidemic potential.

Figure 6

Clostridium difficile PCR ribotype diversity among patients with a confirmed diagnosis of *C. difficile* infection in the study, by age group (596 isolates from 595 CDI cases), EUCLID, 2012–13 and 2013[a]



EUCLID: European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea.

The charts show the proportion of most common ribotypes per age group; the percentages are based on the number of typed isolates.

Data from the following countries (n = 19) were included: Austria, Belgium, Bulgaria, Czech Republic, Finland, France, Germany, Greece, Hungary, Ireland, Italy, the Netherlands, Poland, Portugal, Romania, Slovakia, Spain, Sweden and United Kingdom. None of the faecal samples submitted from Slovenia during the two sampling days were found to be positive for *C. difficile* or its toxins.

[a] The countries submitted inpatient diarrhoeal samples on two sampling days (one day in winter, in December 2012 or January 2013, and one day in summer, in July or August 2013).

Effect of patient age on *C. difficile* PCR ribotype distribution

A total of 596 *C. difficile* PCR ribotypes were identified from the faecal samples of 595 CDI-positive patients, aged 1–99 years, in the study. To investigate if ribotype diversity and distribution varied according to patient age, we analysed the ribotype distribution in four patient age groups: 2 to <18 years (n=18), 18 to <65 years (n=144), ≥65 years (n=412) and ≥81 years (n=195). As the number of patients aged 2 to <18 years was small, the combined age group of 2 to < 65 years is shown (Figure 6). The ribotypes of isolates from samples of patients aged under 2 years were not included in the analysis, as the role of *C. difficile* in infants is uncertain. We found that the number of unique ribotypes identified increased with patient age.

When comparing two age groups with similar patient numbers, 39 individual ribotypes were isolated in patients aged 18 to < 65 years, while 59 were identified in patients ≥ 81 years. Analysis of Simpson's reciprocal index of diversity showed that overall ribotype diversity was higher in patients aged ≥ 81 years (Simpson's reciprocal index: 21.16) than in those aged 18 to < 65 years (Simpson's reciprocal index: 10.1).

Ribotype 001/072 was commonly found in all age groups, with no obvious differences in distribution according to patient age (13% (n = 19/144) in CDI cases aged 18 to < 65 years, 10% (n = 42/412) in ≥ 65 year-olds and 14% (n = 27/195) in ≥ 81 year-olds). Other commonly isolated ribotypes, such as 014/020 (11% (n = 16/144), 8% (n = 32/412) and 9% (n = 18/195), respectively) and 078 (3% (n = 5/144), 3% (n = 13/412) and 3% (n = 6/195), respectively) were also consistently found in all patient age groups, with no noticeable age-associated variation in prevalence (patients aged 2 to < 18 years were excluded from this analysis due to the small sample size).

Ribotype 027 occurred in all patient age groups but we observed considerable variation in prevalence. While this ribotype accounted for 22% (n = 32/144) of CDI cases aged 18 to < 65 years, the prevalence was significantly lower in those aged ≥ 65 years (14%, n = 59/412) and was significantly further decreased in those aged ≥ 81 years (9%, n = 18/195) (chi-squared test p = 0.001).

To exclude any bias from country-specific variation in our analysis, we investigated the differences in ribotype distribution in CDI cases aged 18 to < 65 years (n = 51) and ≥ 65 years (n = 172) from participating hospitals in Germany (the largest country in the study). We observed a similar trend, with the prevalence of ribotype 027 in patients aged 18 to < 65 years (33%, n = 17/51) double that of the prevalence in those aged ≥ 65 years (15%, n = 26/172).

We observed that of 117 isolated *C. difficile* ribotypes from patients aged under 2 years, only 22 (18.8%) were associated with a positive test result for detection of free *C. difficile* toxin in the stool sample. By contrast, 26.5% (18/68) of ribotypes isolated from patients aged 2 to < 18 years, 48.6% (144/296) from those aged 18 to < 65 years, 57.6% (412/712) from those aged ≥ 65 years and 60.6% (195/322) from those aged ≥ 81 years were associated with positive test results; the differences in the rates of toxin-positive test results among these age groups were statistically significant (p < 0.001). This finding supports the view that detection of *C. difficile* in infants and neonates often reflects asymptomatic colonisation.

Interestingly, ribotype 356 was commonly isolated in those aged under 2 years (4/22) but was rarely seen in those ≥ 65 years (2%, 7/412) and was not found at all in patients aged 2 to < 65 or ≥ 81 years. This suggests that ribotype 356 may be more commonly found in infants and neonates than older patients, although more data from a larger sample are required to verify this observation.

Discussion

The findings of this EUCLID analysis highlight the changing epidemiology of *C. difficile* in Europe. We found an increase in overall ribotype diversity, with more than double the number of ribotypes identified in this study compared with data from 2008 [11]. It is important to note, however, that the possible suboptimal testing methodology and selection of cases for isolate ribotyping in the previous study, in addition to the smaller sample size, would likely have led to an under-representation of the recorded strain diversity. Nevertheless, notably, the prevalence of ribotype 027 had increased more than threefold (from 5% to 18.6%) since 2008 [11] and 027 was the most commonly isolated ribotype in the participating European countries in our study (on two sampling days in 2012–13 and 2013).

No clear difference in ribotype distribution was observed when samples that tested positive for free *C. difficile* toxin were compared with those that tested negative . This suggests that there is no discernible difference in those ribotypes causing *C. difficile* disease and those involved with colonisation, at least in inpatients with diarrhoea. The toxin component of the testing algorithm used in our study has been reported to have a sensitivity of 67.3% in the combined test and 84.3% as a single assay [21,22]. Thus, some patients classified here as likely colonised will in fact have been missed CDI cases.

Some similarities with the 2008 study [11] were observed, with ribotype 001/072 and ribotype 014 remaining highly prevalent among *C. difficile* clinical isolates across many European countries. However, we observed an almost threefold reduction in the prevalence of ribotype 078 in this study compared with that in 2008 [11]. Also, ribotype 106, which was associated with 26% and 20% of CDI cases in England in 2005 [23] and 2007–08 [24], respectively, was not found at all in the UK study hospitals in our study and accounted for only 0.6% of all *C. difficile* isolates in Europe. Ribotype 027 previously accounted for 55% of isolates in England in 2007–08 [25], but only represented 2.3% of UK isolates in the present study.

Our previous analysis showed a clear shift in ribotype 027 endemicity, from the UK and Ireland in 2008 to Germany, Hungary, Poland and Romania on the two sampling days in 2012–13 and 2013 [12]. The reason for a shift in ribotype 027 prevalence towards Germany and Eastern Europe is not clear, but may have been influenced by national CDI testing policies. Our earlier analysis identified an inverse correlation between the rate of CDI testing and prevalence of ribotype 027 across Europe [12]. Thus, an increased awareness of

CDI, via the use of optimum diagnostic tests, may have permitted the implementation of more timely infection prevention and antimicrobial prescribing interventions in Northern Europe, and so better control of epidemic strains such as ribotype 027 in this region since 2008 [12].

The substantial variation in ribotype distribution observed among study countries and regions is in keeping with the results of the 2008 study, in which the most commonly isolated *C. difficile* ribotypes were found in many countries across Europe and the geographical distribution of some ribotypes suggested regional spread [11]. Our findings highlight the diverse epidemiology of *C. difficile* across Europe. We observed that a high prevalence of ribotypes 027 and 176 was associated with low overall country-specific ribotype diversity, which is perhaps unsurprising. Countries with CDI outbreaks caused by epidemic strains such as ribotypes 027 and 176 would likely have high incidence rates but lower overall diversity due to more healthcare-associated transmission of dominant ribotypes. This scenario may have been seen first-hand in the UK, where CDI incidence rapidly increased in 2006 with the emergence of ribotype 027 [23-25]. Subsequent infection control measures and antibiotic stewardship may have since shifted the UK into an endemic scenario with high ribotype diversity and a low prevalence of ribotype 027 [26].

We found no correlation between ribotype 001/072 prevalence and overall ribotype diversity, suggesting that ribotypes 027 and 176 may be more successful at outcompeting such other ribotypes with epidemic potential. The drivers for dominant ribotypes in particular countries, for example ribotype 176 in the Czech Republic and ribotype 018 in Italy, are not yet known. In the UK, the practice of restricting precriptions of cephalosporins and fluoroquinolones since 2009 has been associated with falling prevalence of ribotype 027, which suggests that reduced selection of antibiotic-resistant (in this case, fluoroquinolones) *C. difficile* clones could be a key control measure [25,27]. Future studies of comparative fitness among different *C. difficile* ribotypes would be of particular interest.

The observed differences in ribotype distribution for CDI cases among patient age groups also suggest that some ribotypes may be more likely to cause CDI. Of note was the significant reduction in ribotype 027 prevalence with increasing patient age, which is perhaps at odds with the known poor clinical outcomes associated with this strain type in elderly patients [28]. It is possible that our data reflect differences in *C. difficile* selection pressures according to age; for example, less frequent use of high CDI-risk antibiotics in elderly patients [29,30]. Overall ribotype diversity appeared to increase with age, which may be related to the observed inverse correlation between ribotype diversity and 027 prevalence.

We noted the presence of some known non-toxigenic *C. difficile* types among isolates associated with CDI positive tests, with ribotypes 140 (3.7%) and 010 (3.5%) the fourth and sixth most commonly isolated in the participating countries in Europe, respectively. A likely explanation for this finding is that the submitted faecal sample contained more than one ribotype (including toxigenic ribotypes responsible for a positive toxin test) but, when *C. difficile* was cultured, the predominant strain was ribotype 140 or 010. The rate of mixed *C. difficile* genotypes in faecal samples of patients with CDI has ranged from 7% to 13% in previous studies [31-34] and the coexistence of multiple PCR ribotypes has been previously reported as a potential limitation of *C. difficile* epidemiological studies [35]. In our study, several single *C. difficile* colonies were pooled before DNA extraction and, while this method allows accurate identification of the predominant ribotype, not every ribotype present within the sample can be identified. Therefore, in some cases the relative abundance of the disease-causing ribotype may have been too low for identification.

The findings of this analysis from EUCLID emphasise the importance of continuous national and European surveillance programmes to monitor the dynamic epidemiology of *C. difficile*, including use of optimal diagnostic methods to identify CDI cases. Further studies are also necessary to better understand how *C. difficile* ribotype distribution varies among patient populations, and factors contributing to an observed shift of ribotype 027 to Germany and Eastern Europe.

EUCLID study group

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Conflict of interest

HA and GD: none declared.

MHW has received grant support/consultancy fees/honorarium from Astellas. KAD has received honoraria from Astellas. CML and DAB are employees of Astellas Pharma EMEA.

Authors' contributions

The EUCLID study was designed by KAD, MHW and CML with support from the EUCLID core group and on behalf of the EUCLID study group. GLD was responsible for project management and sample logistics. HA performed PCR ribotyping analyses. KAD, DAB, CML and MHW analysed data and wrote the report. All authors reviewed drafts of the report.

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SURVEILLANCE AND OUTBREAK REPORT

Enhanced surveillance of *Clostridium difficile* infection occurring outside hospital, England, 2011 to 2013

WN Fawley [1], KA Davies [2,3], T Morris [4], P Parnell [2], R Howe [3], MH Wilcox [1,2,3], on behalf of the Clostridium difficile Ribotyping Network (CDRN) Working Group [5]

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There are limited national epidemiological data for community-associated (CA)-*Clostridium difficile* infections (CDIs). Between March 2011 and March 2013, laboratories in England submitted to the *Clostridium difficile* Ribotyping Network (CDRN) up to 10 diarrhoeal faecal samples from successive patients with CA-CDI, defined here as *C. difficile* toxin-positive diarrhoea commencing outside hospital (or less than 48 hours after hospital admission), including those cases associated with community-based residential care, with no discharge from hospital within the previous 12 weeks. Patient demographics and *C. difficile* PCR ribotypes were compared for CA-CDIs in our study and presumed healthcare-associated (HA) CDIs via CDRN. Ribotype diversity indices, ranking and relative prevalences were very similar in CA- vs HA-CDIs, although ribotypes 002 ($p \leq 0.0001$),020 ($p = 0.009$) and 056 ($p < 0.0001$) predominated in CA-CDIs; ribotype 027 ($p = 0.01$) predominated in HA-CDIs. Epidemic ribotypes 027 and 078 predominated in institutional residents with CDI (including care/nursing homes) compared with people with CDI living at home. Ribotype diversity decreased with increasing age in HA-CDIs, but not in CA-CDIs. Ribotype 078 CA-CDIs were significantly more common in elderly people (3.4% (6/174) vs 8.7% (45/519) in those aged < 65 and ≥ 65 years, respectively; $p = 0.019$). No antibiotics were prescribed in the previous four weeks in about twofold more CA-CDI vs HAs (38.6% (129/334) vs 20.3% (1,226/6,028); $p < 0.0001$). We found very similar ribotype distributions in CA- and HA-CDIs, although a few ribotypes significantly predominated in one setting. These national data emphasise the close interplay between, and likely common reservoirs for, CDIs, particularly when epidemic strains are not dominant.

Introduction

Clostridium difficile infection (CDI) has long been considered primarily to be a nosocomial disease, most notably associated with increased age, hospitalisation and antibiotic use [1]. There is, however, limited information on the epidemiology of community-associated (CA)-CDI, but data suggest that the incidence of CA-CDI could be increasing [2-4]. However, variation in reported rates may be due to varying definitions and case ascertainment bias as a consequence of suboptimal or incomplete testing of community-based patients [5]. In general, it is also known that there is marked underascertainment of the causes of diarrhoea in the community [6,7].

In conjunction with mandatory reporting of CDI cases in England [8], additional surveillance includes voluntary submission of faecal samples to a centrally funded scheme (*Clostridium difficile* Ribotyping Network (CDRN) for England and Northern Ireland), which has provided specific data on circulating *C. difficile* PCR ribotypes since 2007. CDRN now examines over a third of all reported CDI cases in England [9]. A better understanding of the epidemiology of CA-CDI is required in order to achieve improved prevention and control of cases. We have therefore augmented the national CDRN surveillance scheme to compare the patient demographics and *C. difficile* ribotypes associated with healthcare (HA)- and CA-CDI over a two-year period, March 2011 to March 2013.

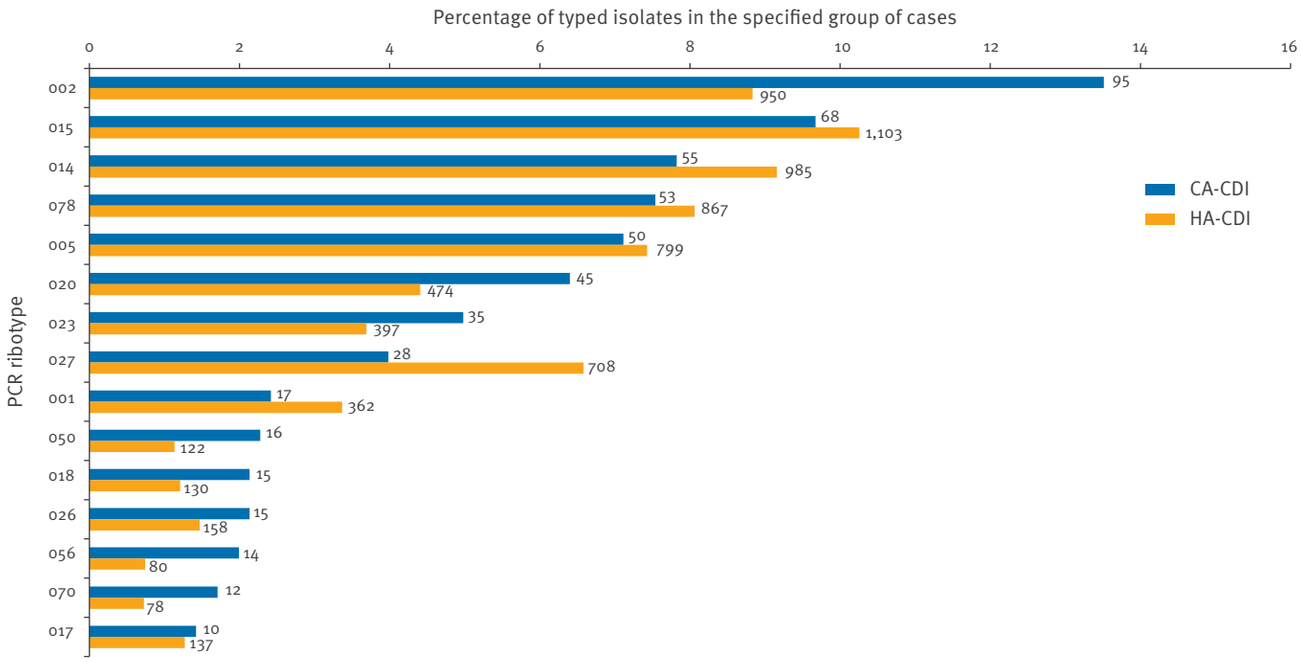
Methods

Community-associated-*C. difficile* infection surveillance scheme

During March 2011 to March 2013, hospital microbiology laboratories in England were asked to send up to 10 faecal samples to their regional CDRN laboratory from successive CDI cases who met the definition of CA-CDI: *C. difficile* toxin-positive diarrhoea (loose stools with no clear medical/surgical explanation) with onset of symptoms while outside hospital (or within the first 48 hours of hospital admission), including those cases associated with community-based residential care, and

Figure 1

Top 15 *Clostridium difficile* PCR ribotypes from cases of community-associated *C. difficile* infection (n = 703) and hospital-associated *C. difficile* infection[a] (n = 10, 754), England, March 2011–March 2013



CA: community-associated; CDI: *Clostridium difficile* infection; HA: hospital-associated.

Ribotype proportions are expressed as percentages of the total number of ribotyped *C. difficile* isolates from cases within the CA-CDI and HA-CDI datasets. The number of isolates of each ribotype are indicated at the end of the bars.

[a] HA-CDI data were obtained from the *Clostridium difficile* Ribotyping Network (CDRN).

those without discharge from hospital within the previous 12 weeks [10,11]. More than 90% of (about 150) laboratories were following national (two-stage testing) guidance for CDI diagnosis. All faecal samples submitted were accompanied by a brief patient-based questionnaire (anonymised) that was completed at the local microbiological testing laboratory. The questionnaire recorded demographic data, details of hospitalisation, residency in a care/nursing home, and antibiotic exposure (from patient records where available). Only the first half of the patient's residential post code was collected to permit potential geographical mapping, while retaining anonymity. *C. difficile* was cultured at the receiving CDRN laboratory. If the sample was *C. difficile* culture-negative then another case was recruited prospectively. All *C. difficile* isolates were centralised at the CDRN Reference Laboratory in Leeds, England, and referred to the UK Anaerobe Reference Unit (UKARU) in Cardiff, Wales, for PCR ribotyping. Demographic and typing data were analysed at the CDRN Reference laboratory, Leeds.

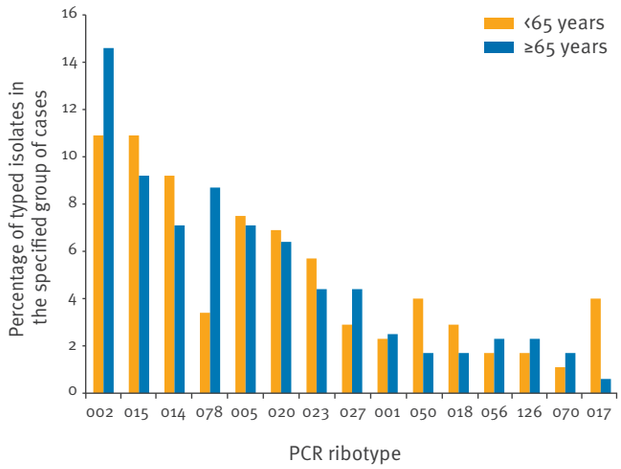
C. difficile culture, identification and PCR ribotyping

C. difficile isolates were recovered from faecal samples at by culture on modified Brazier's cycloserine-cefoxitin-egg yolk agar (Laboratory M, Bury, United Kingdom (UK)) without egg yolk and supplemented with 5 mg/L lysozyme (CCEYL) for 48 hours at 37°C in an anaerobic atmosphere. *C. difficile* isolates were identified by their characteristic smell and colony morphology, fluorescence under long-wave UV light and a latex agglutination test for *C. difficile* somatic antigen (Oxoid Ltd, Basingstoke, UK).

PCR ribotyping was performed at UKARU as described previously [12]. Briefly, DNA was extracted from overnight cultures of *C. difficile* using Chelex 100 resin (BioRad, Hemel Hempstead UK). The 16S-23S intergenic spacer regions were amplified using primers P3: 5'-CTG GGG TGA AGT CGT AAC AAG G-3' and P5: 5'-GCG CCC TTT GTA GCT TGA CC-3'. DNA fragments were concentrated before electrophoresis and resolved using 3% Metaphor agarose (Cambrex Bioscience, Rockland, United States (US)).

Figure 2

The 15 most frequently identified *Clostridium difficile* PCR ribotypes from cases of community-associated *C. difficile* infection by age (< 65 years (n = 174) and ≥ 65 years (n = 519)), England, March 2011–March 2013



Ribotype proportions are expressed as percentages of the total number of ribotyped *C. difficile* isolates from cases who were under 65 years-old and those aged 65 years or more.

Cases of healthcare-associated *Clostridium difficile* infection

Comparative data for presumed HA-CDI cases (onset of symptoms ≥ 48 hours after admission to a healthcare facility or with onset of symptoms in the community within 12 weeks following discharge from a healthcare facility) [10,11] occurring during the same period were obtained from the results of routine CDRN testing. *C. difficile* culture and ribotyping was performed at regional CDRN laboratories, with data collated by the CDRN Reference Laboratory in Leeds. In order to check the accuracy of the classification of routine CDRN cases as HA-CDI, demographic data were collected for all submitted samples in one region (Yorkshire and Humber).

Statistical methods

Univariate analyses were used to compare differences between categories using chi-squared test or Fisher's exact test (where sample size was small, i.e. less than 5, or less than 10 if only one degree of freedom). Median ages were compared by Mann–Whitney test. Ribotype diversity within groups was assessed using Simpson's index, with 95% confidence intervals (CIs) demonstrating variance within groups. Univariate analyses were performed using SPSS version 19, and diversity analyses using PAST version3.

Results

A total of 113 laboratories across England, all serving both hospitals and the community, referred 703 *C. difficile* toxin-positive (and *C. difficile* culture-positive) faecal samples from individual CA-CDI cases between March 2011 and March 2013 (i.e. median of six samples per laboratory, range: 1–25). The collected samples were approximately equally distributed over the two-year period. A dataset of 11,479 CDRN records, for the same period, were used as presumed HA-CDI cases for comparison with CA-CDI cases. CA-CDI cases were predominantly female, elderly (≥ 65 years of age) and resident in their own home (Table).

The most frequently identified ribotype causing CA-CDI was RT002 (95/703; 13.5%) (Figure 1).

CA-CDI cases were significantly more likely than HA-CDIs to be due to ribotype 002 ($p \leq 0.0001$). Although not as commonly isolated, ribotypes 020 and RT056 were also significantly more likely to be found in CA-CDI cases than in HA-CDI cases ($p = 0.009$ and < 0.0001 respectively). Ribotypes known to be associated with enhanced pathogen virulence and poor clinical outcome (078 and 027) were fourth and eighth most frequently identified ribotypes in CA-CDI cases, respectively. Notably, ribotype 027 was found significantly more often in HA-CDI cases than in CA-CDI cases ($p = 0.01$). With the exceptions noted above, comparison of ribotypes causing CA- and HA-CDI showed a very similar ranking and prevalence distribution (Figure 1).

Cases referred to the national CDRN service (additional surveillance in conjunction with mandatorily reported CDI cases) were presumed to represent HA-CDIs. As these could conceivably contain CA-CDIs, however (for example, examined as part of outbreak investigations), we sought to compare the ribotype prevalences for CDRN-referred cases from one region in England (Yorkshire and Humber), comprising 14 distinct hospitals, with known low-level community-based testing, with those for the remainder of the CDRN-referred cases in England during the same study period. All ribotype frequency pairs were within plus or minus 1.9% of each other, with the exception of ribotype 027 (6.6% (708/10,754) CDRN England, 17.8% (265/1,489) CDRN Y and H); this discrepancy was due to hospital-based outbreaks of 027 in the Y and H region.

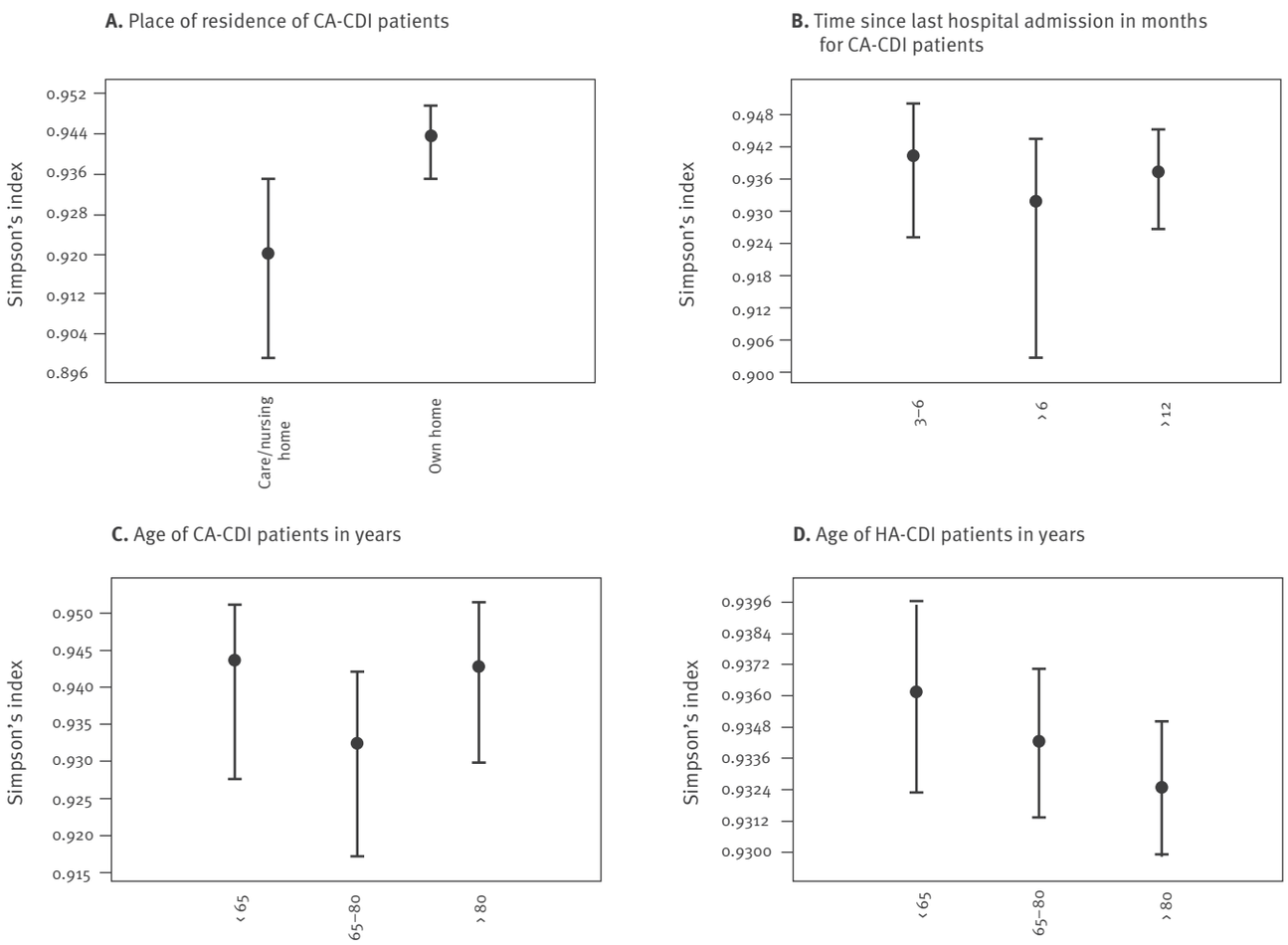
Age of cases

Three quarters of the CA-CDI cases (519/693) were aged ≥ 65 years. Frequencies of the most prevalent ribotypes (top 15) found in the study are shown with respect to patient age in Figure 2.

The prevalence of ribotype 078 in cases of CA-CDI was significantly higher in elderly patients (3.4% (6/174) vs 8.7% (45/519) in those aged < 65 vs ≥ 65 years, respectively; $p = 0.019$). Similarly, ribotype 027 prevalence increased from 2.9% (5/174) to 4.4% (23/519) in elderly patients with CA-CDI, rising further to 5.6% (15/269) in those over 80 years of age, although this trend was not statistically significant. Proportions of cases with CA-CDI with ribotype 002 were found to increase with age, but again this was not statistically significant.

Figure 3

Diversity of *Clostridium difficile* PCR ribotypes (Simpson's indices) for cases of community-associated *C. difficile* infection by (A) place of residence (n = 650), (B) time since last hospital admission (n = 627), (C) age (n = 693), and (D) for cases of hospital-associated *C. difficile* infection by age (n = 10,041)[a], England, March 2011–March 2013



CA: community-associated; CDI: *Clostridium difficile* infection; HA: hospital-associated.

The whiskers represent the 95% confidence intervals around the point estimate of the index.

[a] Hospital-associated *C. difficile* infection data were obtained from the Clostridium difficile Ribotyping Network (CDRN).

Conversely, although numbers were small, proportions of ribotypes 050, 018 and 017 were relatively larger in patients younger than 65 years than in patients 65 years and older (4.0% (7/174), 2.9% (5/174) and 4.0% (7/174) vs. 1.7 (9/519), 1.7 (9/519) and 0.6% (3/519)) respectively. However, none of these were statistically significant (Figure 2).

Median ages of cases with a particular ribotype were generally comparable for CA- and HA-CDI patients. Notably, although numbers were small, cases with CA-CDI due to ribotype 017 infection tended to be younger than corresponding HA-CDI patients (56.5 years and 75 years, respectively; p = 0.13).

Diversity of ribotypes decreased with increasing age in HA-CDI patients, while CA-CDI patients showed no such trend (Figure 3).

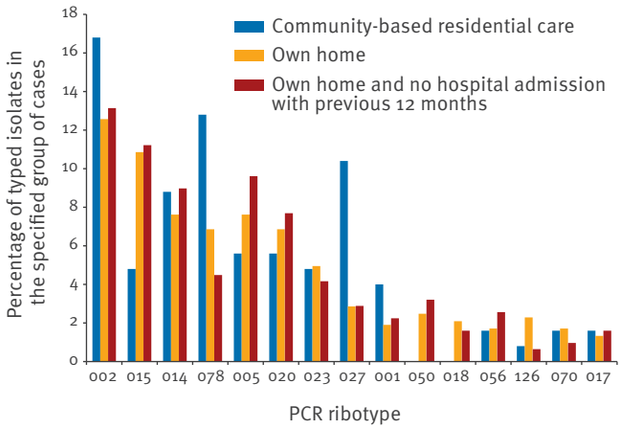
Place of residence

A fifth of the CA-CDI cases (125/525) in the study were associated with community-based residential care. Frequencies of the most prevalent ribotypes (top 15) found in the study with respect to patient residency and recent hospital admission are shown in Figure 4.

Patients with CA-CDI who were living in their own home and had no demonstrable hospital admission within the previous 12 months were classified as having no institutional or healthcare contact. Patients not residing in

Figure 4

Top 15 *Clostridium difficile* PCR ribotypes from cases of community-associated *C. difficile* infection by place of residence (community-based residential care) (n = 125) or their own home (n = 525), or their own home and no hospital admission with the previous 12 months (n=312), England, March 2011–March 2013



Ribotype proportions are expressed as percentages of the total number of ribotyped *C. difficile* isolates from patients residing in a care/nursing home, those residing in their own home, and those residing in their own home with no evidence of hospital admission with the previous 12 months.

their own home were classified as having institutional contact. Larger proportions of ribotypes 002, 078, 027 and 001 were found among patients with institutional contact. Notably, prevalences of ribotypes 027 and 078 were significantly higher in patients with institutional contact compared with those with no contact (10.4% (13/125) vs 2.9% (9/312) and 12.8% (16/125) vs 4.5% (14/312), respectively; both $p<0.001$). Conversely, ribotype 015 was identified significantly more often in patients with no institutional contact versus those with institutional contact (11.2% (35/312) vs 4.8% (6/125), respectively; $p=0.034$). Similar (but non-statistically significant) trends were also observed for ribotypes 005 and 020 CDIs. Although numbers were small, it was interesting to note that ribotypes 050 and 018 were completely absent in CA-CDI patients not residing in their own home.

The diversity of ribotypes associated with CA-CDI cases residing in their own homes per se, was markedly higher than that associated with care/nursing home residence, although this difference was not statistically signficant (Table,Figure 3).

Previous hospital stay

A quarter of CA-CDI cases (158/627) were identified as having been admitted to hospital within the previous three to six months. Frequencies of the most prevalent ribotypes (top 15) from cases in the study with respect to previous hospital stay are shown in Figure 5.

Proportions of CA-CDIs caused by ribotypes 078, 020, 023 and 027 in patients with hospital admission within the previous three to six months were higher than in those with no evidence of hospital admission within the previous year, although these differences were only significant for ribotype 078 (12.0% (19/158) vs 4.5% (18/396); $p=0.005$). Frequencies of several ribotypes, notably 002, 015 and 005, were found to be higher among patients who had no evidence of hospital admission within the previous year as compared with those admitted in the previous three to six months; only the difference in proportions of ribotype 005 was significant (9.8% (39/396) vs 2.5% (4/158); $p=0.003$). Ribotype diversity was similar for CA-CDI cases with no evidence of hospital stay within the previous year compared with those admitted in the previous three to six months (Table, Figure 3).

History of antibiotic use

History of antibiotic use was the most poorly completed part of the CA-CDI case questionnaires (47.5% (334/703) completed). For those with available antibiotic history data, CA-CDI cases were significantly more likely not to have received any antibiotics in the four weeks before their CDI episode when compared with HA-CDI cases (CDRN data) (38.6% (129/334) vs 20.3% (1,226/6,028); $p<0.0001$).

The three most common antibiotics/classes associated with CA-CDI cases were amoxicillin/clavulanic acid (16%; n = 61), amoxicillin/ampicillin (13%; n = 51) and cephalosporins (6%; n = 23); 4% (n = 14) had received a fluoroquinolone. Notably, these data do not take into account the relevant frequencies of antibiotic prescribing.

Frequencies of the most prevalent ribotypes (top 15) found in the study with respect to recent antibiotic use are shown in Figure 6.

A significantly higher proportion of ribotype 050 was associated with antibiotic use (0.78% (1/129) vs 5.8% (12/205); $p=0.013$). For all other comparisons, p was greater than 0.05.

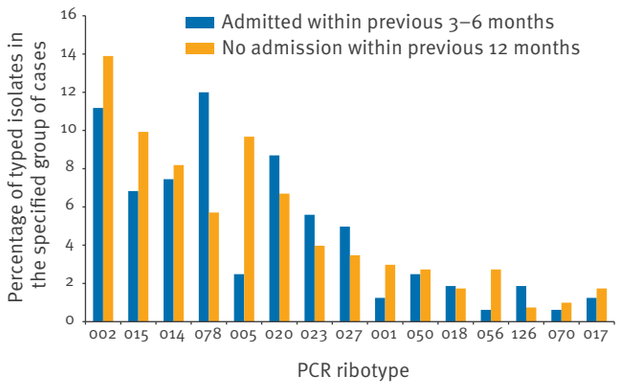
Patients with no institutional or healthcare contact and who did not receive any antibiotics in the previous four weeks, were classified as having no established risk factors for CDI. The prevalence of ribotype 002 was higher in those patients with no established risk factors when compared with those with at least one known risk factor, although this was not statistically significant (14.5% (9/62) vs 12.5% (52/415); $p=0.662$).

Discussion

To the best of our knowledge, this is the first large study in the UK to compare the epidemiology of CA- vs HA-CDI. In marked contrast to earlier reports, when HA-CDI was closely associated with a small range of epidemic ribotypes [1,13,14], we found very similar ribotype diversity indices for CA- and HA-CDI.

Figure 5

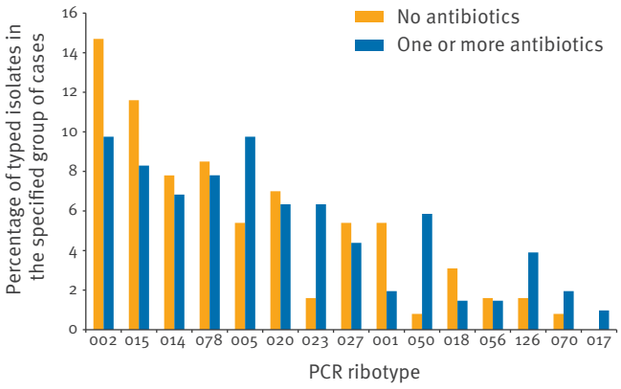
Top 15 *Clostridium difficile* PCR ribotypes from cases of community-associated *C. difficile* infection by status of previous hospital admission (within 3–6 months of their *C. difficile* infection episode (n = 158) and those with no record of hospital admission within the previous 12 months (n = 396)), England, March 2011–March 2013



Ribotype proportions are expressed as percentages of the total number of ribotyped C. difficile isolates from patients admitted to hospital within the previous three to six months and those with no evidence of hospital admission within the previous 12 months.

Figure 6

Top 15 *Clostridium difficile* PCR ribotypes from cases of community-associated *C. difficile* infection by history of antibiotic use during 4 weeks before their *C. difficile* infection episode (no antibiotics (n = 129) and one or more antibiotics (n = 205)), England, March 2011–March 2013



Ribotype proportions are expressed as percentages of the total number of ribotyped *C. difficile* isolates from patients who received no antibiotics and those who received one or more antibiotics.

Furthermore, the ranking and relative prevalences of ribotypes causing CA- and HA-CDIs were very similar. A relatively recent landmark study, using highly discriminatory whole genome sequencing (WGS), showed that the majority of CDIs occurring between September 2007 and March 2011 across a region in England did not represent case-to-case transmission of *C. difficile* [15]. Importantly, in that study, the rate of appearance of new, distinct *C. difficile* genotypes causing infections was constant, suggesting the existence of a large reservoir(s) of *C. difficile*. If correct, this would tend towards a similar distribution of ribotypes causing HA- and CA-CDI, (as found in this study) assuming that there are no powerful selection pressures or niches for particular ribotypes that could promote CDIs in one setting versus the other.

In England, a pragmatic definition has been used in national surveillance to apportion CDI cases between hospitals (symptom onset after 72 hours following admission) and the community (symptom onset in the community or within the first 72 hours following admission to hospital) [8]. However, this definition may exaggerate numbers of cases with apparent CA-CDI as it fails to take into account recent previous hospital admission. Multiple, often large outbreaks were typical around the peak incidence of CDIs in the UK in 2007–08; since then there has been a ca 70–80% decrease in case frequency [13,14] This followed intensive public health campaigns that included multiple infection prevention and control measures designed to reduce transmission of *C. difficile* and alter prescribing of antimicrobials [16]. One of the most striking aspects of this control programme was the substantial decrease in prevalence of ribotype 027 CDIs. In 2007–08, this ribotype caused more than 50% of CDIs in England referred to the CDRN; in subsequent 12-month periods the corresponding proportions were 36% (in 2008–09), 22% (in 2009–10), 13% (in 2010–11), and 9% (in 2011–12) [13]. The control of this epidemic strain, which is associated with poor clinical outcome [17,18], has been paralleled by an increased heterogeneity of ribotypes causing CDIs [13]. This observation is also consistent with the similar distributions of strains found to be causing CA-CDIs and HA-CDIs in this study. Earlier studies in Sweden (1998 and 2004) also reported similar distributions of ribotypes among nosocomial and community settings [19,20]. Such data likely reflect the close interplay between hospital and community settings at times of relatively low levels of hospital-based CDI case-to-case transmission.

While *C. difficile* ribotype distributions were similar among cases of CA- and HA-CDI in our study, there were some notable differences. CA-CDI cases were significantly more likely than HA-CDI cases to be due to ribotype 002 and (less commonly) to ribotypes 020 and 056. Conversely, ribotype 027 was found significantly more often in HA-CDI cases than CA-CDI cases. Ribotype 002 is a relatively frequent cause of HA-CDI and is among several other long-recognised ribotypes, including 015, 014, 020 and 078, which have become more common in the UK, concurrent with the demise of epidemic ribotypes such as 027, 106 and 001 [9,13].

Studies have repeatedly demonstrated a lower median age in patients with CA-CDI compared with HA-CDI [19-22]. However, we did not find a statistically significant difference. Age-related differences may be confounded

Table

Patient-based questionnaire data and *Clostridium difficile* PCR ribotype diversity (Simpson's index) for cases of community-associated *C. difficile* infection (n = 703) and cases of healthcare-associated *C. difficile* infection[a] (n = 11,479), England, March 2011–March 2013

Case characteristics Number		Cases of CA-CDI with available data Simpson's index (95% CI)			Cases of HA-CDI with available data			PCR ribotype diversity	
		Total number per category	%	Number	Total number per category	%	CA-CDI	HA-CDI[b]	
Sex	Male	234	701	33	4,855	11,289 For Simpson's, n = 9,812[b]	43	0.94 (0.92–0.95)	0.94 (0.94–0.94)
	Female	467		67	6,434		57	0.94 (0.93–0.95)	0.94 (0.94–0.95)
Age in years	‹65	174	693	25	2,805	11,387 For Simpson's, n = 10,041[b]	25	0.94 (0.93–0.95)	0.95 (0.94–0.95)
	65–80	250		36	3,843		34	0.93 (0.92–0.94)	0.94 (0.94–0.95)
	›80	269		39	4,739		42	0.94 (0.93–0.95)	0.94 (0.94–0.94)
Place of residence	Community-based residential care	125	650	19	NA	NA	NA	0.92 (0.90–0.94)	NA
	In own home	525		81	NA	NA	NA	0.94 (0.94–0.95)	NA
Previous hospital stay, from sample date	Within less than the previous 3–6 months	158	627	25	NA	NA	NA	0.94 (0.93–0.95)	NA
	Within previous 6 to 12 months	73		12	NA	NA	NA	0.93 (0.90–0.94)	NA
	No evidence of hospital stay within previous 12 months	396		63	NA	NA	NA	0.94 (0.93–0.95)	NA
Antibiotics received, within previous 4 weeks	None	129	334	39	1,226	6,028 For Simpson's, n = 5,279[b]	20	0.93 (0.91–0.94)	0.93 (0.93–0.94)
	1	134		40	2,066		34	0.94 (0.93–0.95)	0.94 (0.94–0.94)
	2	48		14	1,411		23	0.94 (0.90–0.95)	0.94 (0.94–0.94
	3 or more	23		7	1,325		22	0.91 (0.86–0.93)	0.94 (0.94–0.95)

CA: community-associated; CDI: *Clostridium difficile* infection; CI: confidence interval; HA: hospital-associated; NA: not available.

[a] HA-CDI data were obtained from the *Clostridium difficile* Ribotyping Network (CDRN).

[b] Simpson's index was calculated where a ribotype result was available.

by ascertainment bias, including testing policy in hospital versus community settings. We speculate also that differences between studies with respect to age may be driven by ribotype distribution in population cohorts. Data from our study showed that infections associated with certain ribotypes (002, 027, 078) were more common in patients aged ≥ 65 years. Notably, CA-CDIs due to ribotype 078 were ca 2.5-fold more likely to affect an individual aged ≥ 65 years. Median ages of CA-CDI cases and HA-CDI cases were very similar for infections due to ribotypes 002, 027 and 078. A Dutch study in 2008 found a significant difference in the median age of CDI cases due to ribotypes 078 and 027 (67.4 vs 73.5 years, respectively) [21]. In our study, although median age was lower for cases due to ribotype 078 (80 years), vs 82 years for ribotype 027, this difference was not statistically significant. Although numbers were small, ribotype 017-associated CA-CDIs were more than three times more likely to affect a younger individual. Additionally, the median age of patients with ribotype 017-associated CDI was significantly lower in CA-CDI patients than in corresponding HA-CDI patients, suggesting that a true association may exist between ribotype 017 infections and the younger patient in a

community setting. We also found that ribotype diversity decreased with increasing age in HA-CDI patients, while CA-CDI patients showed no such trend.

Recent US studies (2013–15) found that about a third of CDI cases were CA-CDIs [3,4,23]. However, the increasing use of nucleic acid amplification tests (NAATs) alone for the diagnosis of CDI may be confounding US data, given the clear potential for large overestimates of CDI incidence by this sensitive but poorly specific diagnostic approach [24]. Indeed, use of NAATs was found to significantly correlate with higher reported CA-CDI incidence [3]. By contrast, at the time of our study, 79% and 94% of UK hospitals in 2011–12 and 2012–13, respectively, were using an optimised method (screening test followed by a toxin test) for CDI diagnosis [24]. In the US, between 2009 and 2011, ca 40% of cases defined as CA-CDI had high-level exposure to healthcare (i.e. surgery, dialysis, emergency or urgent care visit, inpatient care with no overnight stay, or healthcare personnel with direct patient care), despite no hospital admission in the previous 12 weeks [4]. A further ca 40% had low-level healthcare exposure (i.e. an outpatient visit with a physician or dentist). Thus, only ca 20% of CA-CDI cases had no recorded healthcare contact in the previous 12 weeks. Of note, HA-CDI was taken to include cases occurring in nursing homes (and acute care hospitals or long-term acute care hospitals). There is a key issue regarding consistency between studies and healthcare systems concerning definitions of 'nursing homes'. In the US, there are more than 15,000 nursing homes, each averaging over 100 licensed beds [25]. By contrast, care homes in England (about 17,500) with nursing capability (n = ca 4,000) are about half the size of their US counterparts; typically both nursing and residential care are provided within the same facility [26-28]. In England, about 4% (ca 375,000) of the population aged over 65 years live in care/nursing homes, rising to almost 20% of those aged ≥ 85 years. Thus, a sizeable minority of elderly people live in care homes, but determining whether individuals are receiving nursing as opposed to residential care is problematic, given that care needs may fluctuate. Subjects receiving residential care are not receiving healthcare per se, but instead are helped with normal daily living activities. This highlights the dilemma of how best to categorise subpopulations resident in care homes.

A limitation of our study is that we did not ascertain the level of nursing received by CA-CDI cases in care homes. We chose to define CA-CDI cases to include non-hospital-associated cases living in care homes, noting that the great majority of residents in such settings are not receiving nursing care [26-28]. However, by examining subpopulations resident in the community in care homes, we did demonstrate a clear predominance of epidemic ribotypes, notably 027 and 001, in patients with institutional contact compared with those living in their own home. High prevalence of ribotype 027 CDIs in nursing home residents has been reported in Germany in 2012 [29], but data in this setting are limited [30]. Carriage of *C. difficile*, CDI and subsequent transmission of the pathogen are more common in elderly patients [1], and so it is not surprising that (older) patients associated with community-based residential care had a different distribution of ribotypes compared with community residents living in their own home. Furthermore, we found that CDI cases either resident in their own home or with no evidence of hospital stay within the previous 12 months were associated with higher relative diversity indices than either those residing in care homes or admitted to hospital within the previous six months. More simply, patients with less recent contact with hospitals were more likely to be affected by a more diverse range of *C. difficile* strains than those with more recent contact, presumably reflecting a lower risk of contact with epidemic strains. We did not collect information on CDI outbreaks as this was beyond the scope of the study.

While antibiotic exposure is a key risk factor for CDI [1,31-33], our study has again demonstrated that over a third of CA-CDI cases were associated with no recent history of a prescribed antibiotic, as seen in other studies [22,34-37]. Indeed, we found that CA-CDI cases were nearly twice as likely to have had no antibiotics preceding infection than HA-CDI cases ($p < 0.0001$). Certain ribotypes notably 001, 002 and 015 were more commonly associated with patients receiving no antibiotics before their infection. Such data indicate that antibiotic history might be less of a prerequisite for infection with these *C. difficile* ribotypes and alternative factors support the spread of these ribotypes in the community setting. Other risk factors associated with CA-CDI have been extensively reported, including gastric acid suppressants and contact with infants under two years-old [34,38]. However, no data currently exist to associate such factors with CDI due to ribotypes 002 and 015 in the community setting.

There is increasing evidence linking CDI to environmental sources including water and food [39-41]. Although these studies have identified clinically relevant ribotypes, notably including 078, in foodstuffs, foodborne transmission of *C. difficile* has not been demonstrated. For example, we recently found no differences between hospital and community onset of infection, or in food or environmental exposures between ribotype 078 CDI cases and those caused by other ribotypes [42]. However, conditional logistic regression modelling adjusting for age found that ribotype 078 CDI cases were markedly more likely than other cases to report prior antibiotic exposure (odds ratio: 5.1 (95% CI: 1.6–16.3); $p = 0.002$) [42]. More studies employing WGS are needed to understand the significance of community *C. difficile* reservoirs to human disease. This is probably best achieved early as new strains emerge, not least because once established it becomes more difficult to untangle true risk factors from confounding issues. The emergence of ribotype 244 in Australasia is a good example of the use of WGS to map the spread

of this new clone primarily causing CA-CDI, although a proven community reservoir remains elusive [43].

In summary, while there were examples of ribotypes that significantly predominated in CA- or HA-CDIs, we found very similar ribotype diversity indices, ranking and relative strain prevalences in these two groups. Ribotype 002 was associated with CA-CDI, and there was a clear predominance of epidemic ribotypes, notably 027 and 001, in patients associated with community-based residential care compared with those living in their own home. CA-CDI cases were nearly twice as likely to have had no antibiotics preceding infection than corresponding HA-CDI cases during the same period. Our nationally sourced data emphasise the close interplay between hospital and community settings, particularly when there are relatively low levels of hospital-based case-to-case transmission of *C. difficile* and thus less dominance of epidemic *C. difficile* clones.

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Conflict of interest

MHW has received grants from Abbott, Actelion, Alere, Astellas, Biomerieux, Cerexa, Cubist, Da Volterra, European Tissue Symposium, Merck, Sanofi-Pasteur, Summit, The Medicines Company, Qiagen.

WNF, KAD, TM, PP and RH: none declared.

Authors' contributions

WNF, RH and MHW designed the study, with support from the CDRN working group. TM performed PCR ribotyping. PP was responsible for sample logistics and data collection, with support from the CDRN working group in their respective regions. WNF, KAD and MHW analysed data and wrote the report. All authors reviewed drafts of the report.

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Clostridium difficile PCR ribotypes 001 and 176 – the common denominator of *C. difficile* infection epidemiology in the Czech Republic, 2014

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In 2014, 18 hospitals in the Czech Republic participated in a survey of the incidence of *Clostridium difficile* infections (CDI) in the country. The mean CDI incidence was 6.1 (standard deviation (SD):7.2) cases per 10,000 patient bed-days and 37.8 cases (SD: 41.4) per 10,000 admissions. The mean CDI testing frequency was 39.5 tests (SD: 25.4) per 10,000 patient bed-days and 255.8 tests (SD: 164.0) per 10,000 admissions. A total of 774 *C. difficile* isolates were investigated, of which 225 (29%) belonged to PCR ribotype 176, and 184 isolates (24%) belonged to PCR ribotype 001. Multilocus variable-number tandem repeat analysis (MLVA) revealed 27 clonal complexes formed by 84% (190/225) of PCR ribotype 176 isolates, and 14 clonal complexes formed by 77% (141/184) of PCR ribotype 001 isolates. Clonal clusters of PCR ribotypes 176 and 001 were observed in 11 and 7 hospitals, respectively. Our data demonstrate the spread of two *C. difficile* PCR ribotypes within 18 hospitals in the Czech Republic, stressing the importance of standardising CDI testing protocols and implementing mandatory CDI surveillance in the country.

Introduction

Clostridium difficile is the most important bacterial cause of hospital-acquired diarrhoea. Two large studies have been carried out to map and update data on *C. difficile* infection (CDI) in Europe [1,2]. CDI incidence showed an increasing trend: in the first study in 2008, the mean incidence in the participating countries was 4.1 cases per 10,000 patient bed-days [1], while in the second, in 2011–13, it was 7.0 CDI cases per 10, 000 patient bed-days in the countries involved [2].

Results of the 2008 study – a hospital-based survey involving 34 European countries – showed that the Czech Republic had a low incidence of CDI (1.1/10,000 patient bed-days), without the presence of *C. difficile* PCR ribotypes 027 and 176 [1]. Spread of PCR ribotype 027 has been seen worldwide [3] and is known to be associated with hospital CDI outbreaks [4] and severe course of disease and increased mortality [5].

Ribotype 176 is closely related to 027 [6,7] and can be misidentified by commercial tests targeting a single-base-pair deletion at nucleotide 117 in the *C. difficile tcdC* gene [8]. In 2009, shortly afterward the 2008 study, the occurrence of ribotype 176 was reported in certain areas of the country (Eastern Bohemia and Moravia) [9]. This ribotype has persisted in the Czech Republic [8] and was also reported in Poland in 2008–13, which borders the country [10,11].

Results from the second study – involving 20 European countries – revealed an increasing CDI incidence rate in the Czech Republic (4.4 cases in 2011–12/10,000 patient bed-days and 6.2 cases/10,000 patient bed-days in 2012–13) [2].

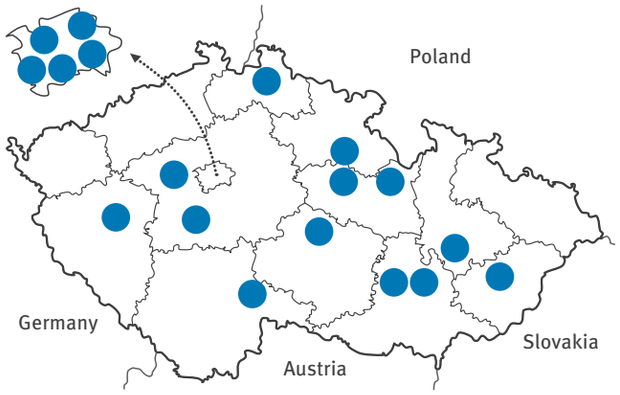
This observation prompted us to determine the CDI incidence in 2014 in a number of hospitals distributed across the Czech Republic (n = 18) and to gain an insight into the prevailing *C. difficile* ribotypes.

Methods

A CDI case was defined as a hospitalised patient (more than two years-old) with both diarrhoea and laboratory confirmation of CDI by a positive test result for the presence of GDH and toxin A/B and/or the detection

Figure 1

Location of hospitals participating in survey of incidence of *Clostridium difficile* infection, Czech Republic, 2014 (n = 18)



of a toxin-producing *C. difficile* strain using toxigenic culture or nucleic acid amplification test (NAAT) in the stool sample.

Testing for CDI was requested by the attending physician based on clinical symptoms indicating CDI (primarily diarrhoea). Hospital-associated and community-associated CDI cases were included in the analysis.

During 2014, hospital microbiology departments of the 18 selected hospitals were asked to send *C. difficile* isolates cultured from stool samples from hospitalised CDI patients to the Department of Medical Microbiology of the University Hospital Motol in Prague.

Mean CDI incidence and CDI testing frequency for all participating hospitals was calculated using the total number of admissions, total number of patient bed-days, number of non-duplicated glutamate dehydrogenase (GDH) and toxin A/B positive tests performed in 2014, using information obtained from the participating hospitals. The hospitals also provided information about their CDI laboratory diagnostic algorithms.

C. difficile isolates were further characterised using PCR ribotyping, detection of the presence of genes for toxin production (*tcdA* (A), *tcdB* (B), *cdtA* and *cdtB* (binary)) by a multiplex PCR [12] and multilocus variable-number tandem repeat analysis (MLVA).

PCR ribotyping based on capillary electrophoresis was performed according to the method described by Stubbs et al. [13]. The results were compared with data in WEBRIBO, a web-based database containing a broad spectrum of uploaded capillary electrophoresis-ribotyping profiles [14], and profiles from an international capillary electrophoresis-ribotyping validation study [15]. The diversity of ribotypes for each hospital was calculated using the Shannon index [16], for which a higher value is an indicator of greater diversity.

For MLVA, five regions with short tandem repeats were sequenced: A6Cd, B7Cd, C6Cd, G8Cd [17] and CDR60 [18], with a change of reverse primer for G8Cd, as described elsewhere [19]. The number of tandem repeats was counted manually after software processing (Sequencing Analysis Software, Applied Biosystems). The sum of tandem repeat differences (STRD) in five loci determines the genetic relatedness of isolates. Minimum spanning trees were created using Bionumerics v5.1 (Applied Maths). A clonal complex was defined as an STRD ≤ 2, a genetically related cluster as an STRD ≥ 3 to ≤ 10 [17].

Results

Participating hospitals

A total of 18 hospitals, covering the country's major regions, voluntarily participated in the survey: seven tertiary care institutions, 10 secondary care facilities and one specialised centre. The size of hospital is indicated by the number of beds in 2014 (Table 1). These 18 hospitals represented about 30% of hospital-bed capacity in the Czech Republic in 2013 [20] (2014 data unavailable). Their location is shown in Figure 1.

Incidence of *C. difficile* infection and testing frequency

The incidence of CDI in 2014 varied from 1.5 to 34.7 (median: 3.9) cases per 10,000 patient bed-days (mean: 6.1 cases (standard deviation (SD): 7.2)/10,000 patient bed-days), and from 11.8 to 201.2 (median: 26.5) cases per 10,000 admissions (mean: 37.8 cases (SD: 41.4)/10,000 admissions).

The frequency of testing for CDI in the hospital laboratories varied from 6.0 to 116.3 tests (median: 28.9) per 10,000 patient bed-days (mean: 39.5 (SD: 25.4) tests per 10,000 patient bed-days), and from 36.4 to 673.5 tests (median: 216.7) per 10,000 admissions (mean: 255.8 tests (SD: 164.0)/10,000 admissions) (Table 1).

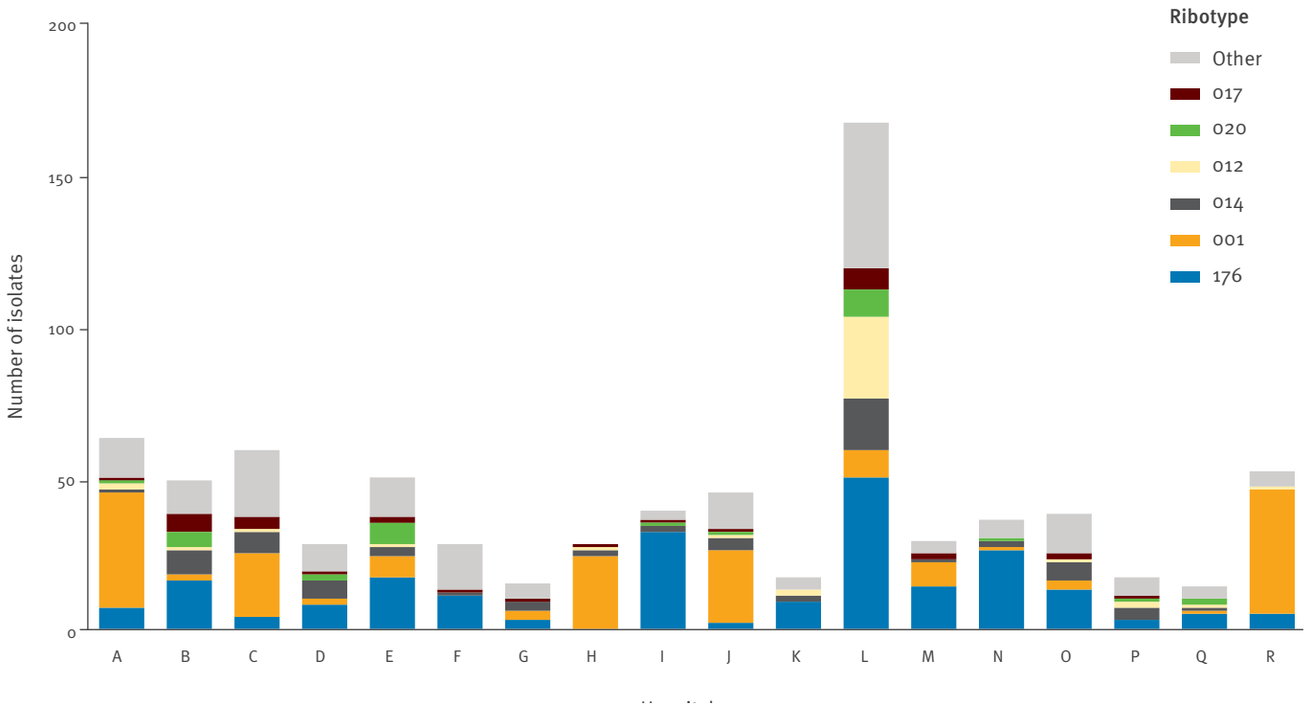
C. difficile infection testing algorithms

Four different CDI testing algorithms were used during the study period (Table 1). All hospitals in the study used the detection of GDH and toxins A/B as the first (screening) part of their testing algorithm: 14 used lateral flow immunoassay (LFIA), three used a chemiluminescent immunoassay (CLIA) and one a chromatographic immunoassay (CIA).

A total of 16 hospitals performed anaerobic culture of GDH-positive and toxin-positive or toxin-negative samples, but only two of these tested toxin production or detected the presence of genes for toxin production of isolated *C. difficile* strains (one by LFIA and one by toxin gene multiplex PCR). The remaining two hospitals, which did not routinely perform anaerobic culture, used PCR detection of the presence of *C. difficile* toxin

Figure 2

Distribution of the six most prevalent PCR ribotypes of *Clostridium difficile* in 18 hospitals participating in survey of incidence of *C. difficile* infection, Czech Republic, 2014 (n = 774)



genes in GDH-positive and toxin A/B-negative stool samples.

Of the 18 hospitals, 10 used a commercial PCR test, eight for rapid diagnosis if requested by the physician. In total, 774 *C. difficile* isolates were available for further analysis in our study: 378 were from male patients (49%) and 396 from female patients (51%). The mean age was 68 years (SD: 20); the median was 72 years (range: 2–101). Of the 774 patients, 537 (69%) were aged 65 years or older.

PCR ribotypes of *C. difficile* isolates

Of the 774 *C. difficile* isolates, 737 (95%) belonged to 33 different ribotypes, and 37 (5%) were defined as new ribotypes, as their electrophoretic profiles differed from each other and did not match any in the WEBRIBO database.

The most frequent PCR ribotype, 176, was found in 225 isolates (29%) in 17 hospitals. The second most frequent, PCR ribotype 001, was identified in 184 isolates (24%) in 14 hospitals. Other frequently found PCR ribotypes were: 014 (n = 70 (9%); 16 hospitals), 012 (n = 41 (5%); 12 hospitals), 020 (n = 31 (4%); 14 hospitals), 017 (n = 30 (4%); 10 hospitals). The distribution of the six most prevalent PCR ribotypes (581 isolates, 75%) within the participating hospitals is shown in Figure 2. Other less frequent ribotypes found were as follows, with the number of isolates per ribotype shown in parentheses: 002 (n=20), 005 (n=14), 081 (n=11), 029 (n=10), 015 (n=10), 070 (n = 9), 023 (n = 8), 078 (n = 7), 003 (n = 6), 503 (n = 5), 449 (n = 5), 046 (n = 5), 018 (n = 5), 087 (n = 5), 049 (n = 5), 126 (n = 4), AI-75 (n = 4), AI-9–1 (n = 4), 054 (n = 4), 446 (n = 3), AI-82/1 (n = 3), 053 (n = 2), 027 (n = 2), AI-60 (n = 2), 043 (n = 1), 236 (n = 1) and AI-12 (n = 1).

The Shannon index, used to determine the diversity of the ribotypes, varied from 0.54 to 2.56. The Shannon index of all the *C. difficile* ribotypes in the study was 2.58, indicating a highly diverse set of *C. difficile* isolates.

Further characterisation of *C. difficile* isolates

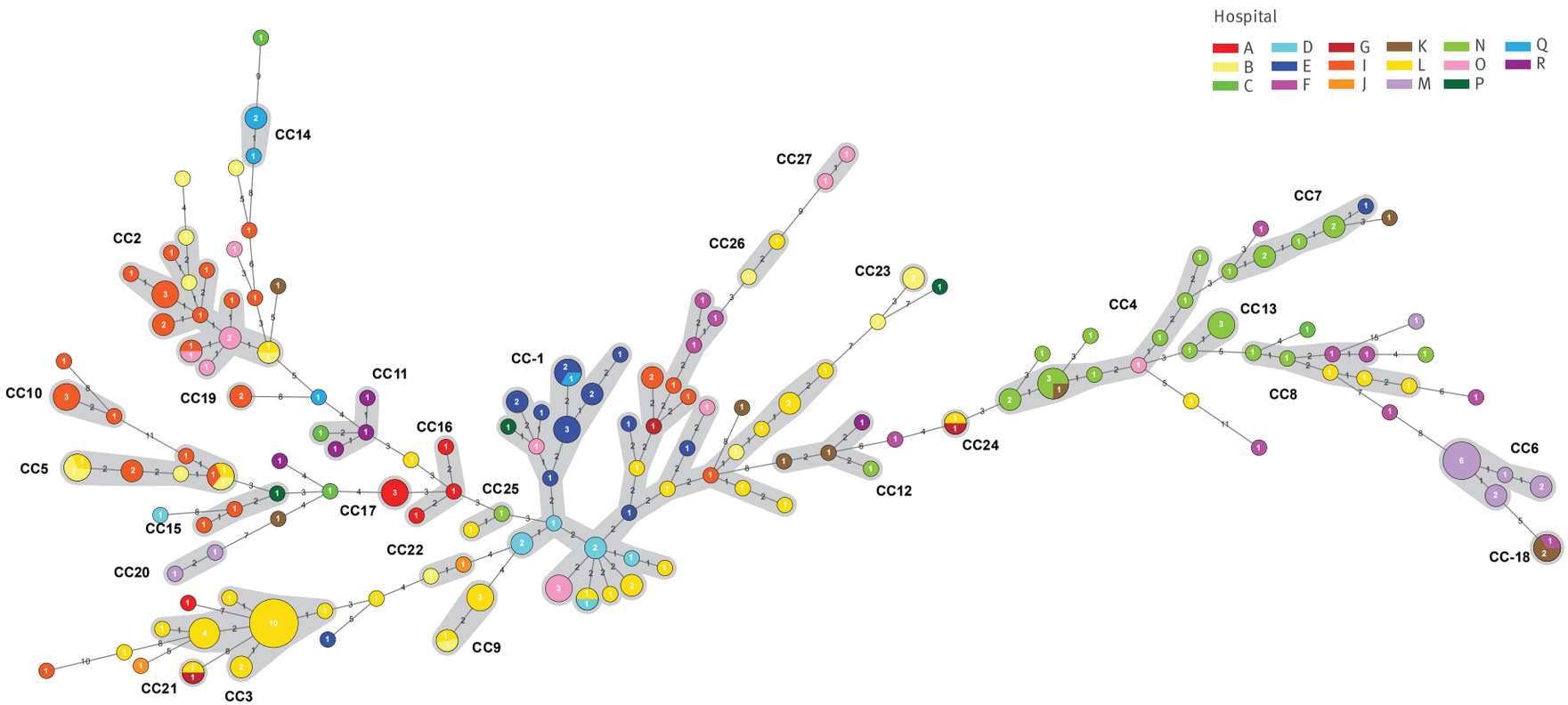
Genes for production of three *C. difficile* toxins (A, B and binary) were detected in 246 (32%) of the isolates belonging to the following PCR ribotypes: 176 (n = 225 (29%)), 023 (n = 8 (1%)), 078 (n = 7 (0.9%)), 126 (n = 4 (0.5%)) and 027 (n = 2 (0.3%)). For the other 528 isolates (68%), only genes for production of toxins A and B were detected.

MLVA of five variable-number tandem repeat loci was performed for the 225 isolates of ribotype 176 and 184 isolates of ribotype 001, and two minimum spanning trees were generated.

In total, 27 clonal complexes comprising 190 isolates (84%) were found in the minimum spanning tree of PCR ribotype 176 isolates (Table 2). For each clonal complex, the number of isolates/number of hospitals in which they were found are shown in parentheses: CC1 (52/10); CC2 (19/4); CC3 (19/1); CC4 (11/3); CC5 (10/3); CC6 (11/1);

Figure 3

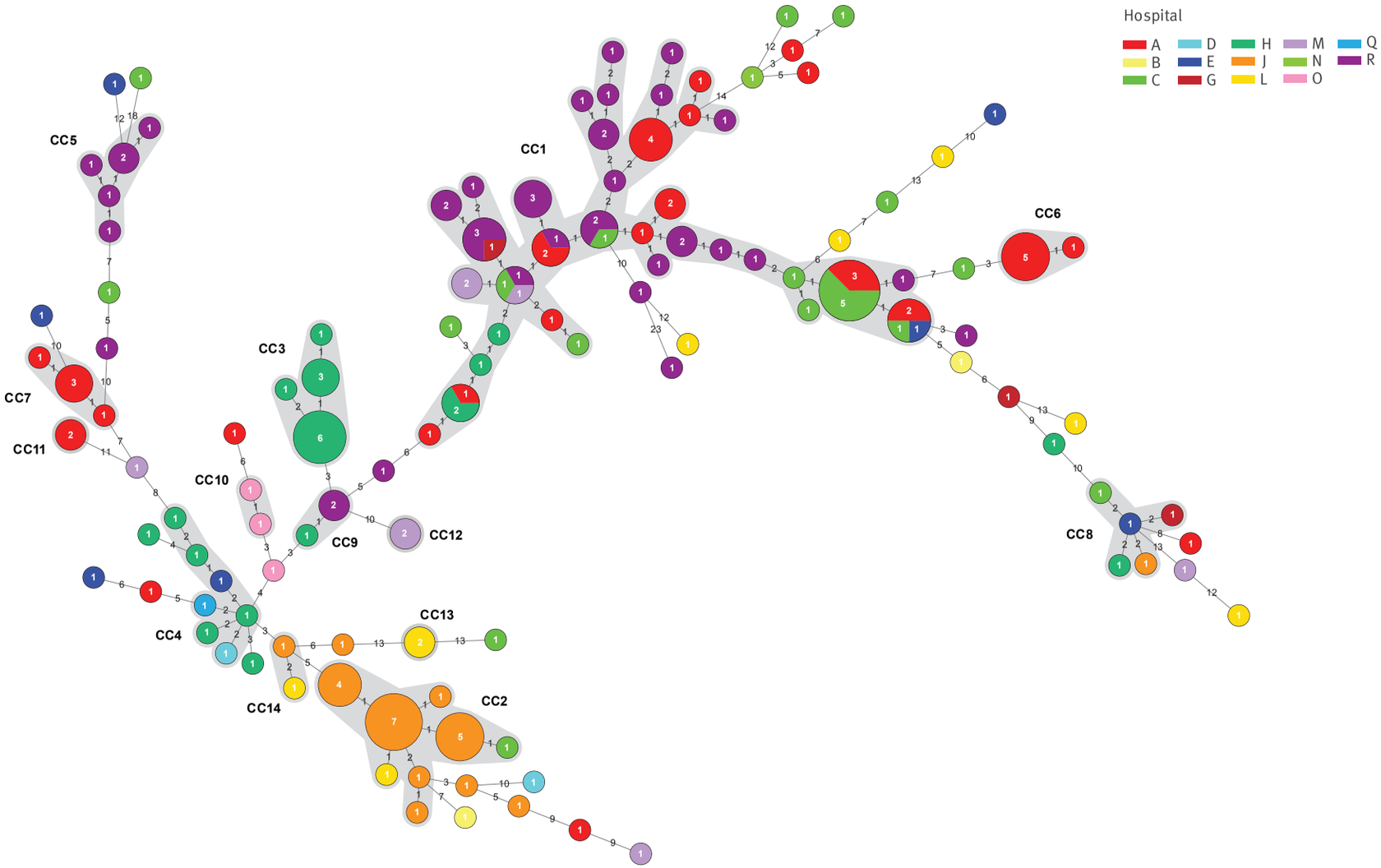
Minimum spanning tree of *Clostridium difficile* PCR ribotype 176 isolates from 17 of 18 hospitals participating in survey of incidence of *C. difficile* infection, Czech Republic, 2014 (n = 225)



Each hospital is represented by a different colour (see key). The numbers in the circles represent the number of *C. difficile* PCR ribotype 176 isolates. If the number is greater than one, it represents the number of isolates with a sum of tandem repeat differences (STRD) = 0 (i.e. 100% identical in five variable-number tandem repeat loci). The numbers on the lines represent the STRD between isolates.

Figure 4

Minimum spanning tree of *Clostridium difficile* PCR ribotype 001 isolates from 14 of 18 hospitals participating in survey of incidence of *C. difficile* infection, Czech Republic, 2014 (n = 184)



Each hospital is represented by a different colour (see key). The numbers in the circles represent the number of *C. difficile* PCR ribotype 001 isolates. If the number is greater than one, it represents the number of isolates with a sum of tandem repeat differences (STRD) = 0 (i.e. 100% identical in five variable-number tandem repeat loci). The numbers on the lines represent the STRD between isolates.

Table 1

Characteristics of hospitals participating in survey of the incidence of *Clostridium difficile* infection, Czech Republic, 2014 (n = 18)

Hospital	Number of beds	Care type	CDI testing algorithm	CDI incidence		Testing frequency per 10,000 bed-days	Testing frequency per 10,000 admissions	Number of isolates (n = 774)	Ribotype diversity[a]
				CDI cases per 10,000 patient bed-days	CDI cases per 10,000 admissions				
A	913	T	LFIA, ANAE	2.7	23.1	29.1	251.3	63	1.60
B	1,001	S	LFIA, TC, NAAT	8.9	53.0	52.5	312.1	49	2.11
C	1,913	T	LFIA, ANAE, NAAT	7.5	45.9	66.3	403.7	59	2.45
D	1,063	T	LFIA, NAAT	1.5	11.8	18.3	139.7	28	2.26
E	550	S	LFIA, ANAE, NAAT	3.4	21.9	6.0	36.4	50	2.22
F	1,368	T	LFIA, ANAE, NAAT	3.5	31.4	55.6	494.2	28	2.31
G	305	SC	LFIA, NAAT	6.2	45.6	71.1	519.4	15	1.96
H	342	S	CLIA, ANAE	6.8	39.3	27.7	159.2	28	0.54
I	531	S	LFIA, ANAE	5.6	29.8	25.8	137.2	39	0.78
J	950	S	LFIA, ANAE	4.1	22.2	28.7	154.6	45	1.90
K	247	S	CLIA, ANAE, NAAT	34.7	201.2	116.3	673.5	17	1.43
L	2,189	T	CLIA, TC, NAAT	2.6	15.7	18.3	111.0	167	2.56
M	1,184	T	LFIA, ANAE	2.5	14.9	17.8	106.6	29	1.47
N	938	S	LFIA, ANAE, NAAT	6.2	39.2	45.4	287.7	36	1.15
O	1,689	T	LFIA, ANAE, NAAT	2.2	14.7	28.7	194.8	38	2.27
P	455	S	CIA, ANAE	2.6	16.5	20.9	134.4	17	2.15
Q	664	S	LFIA, ANAE	3.6	22.3	40.6	250.2	14	1.97
R	962	S	LFIA, ANAE	5.5	31.2	42.0	238.6	52	0.87
Mean (SD)	–	–	–	6.1 (7.2)	37.8 (41.4)	39.5 (25.4)	255.8 (164.0)	–	–
Median (range)	–	–	–	3.9 (1.5–34.7)	26.5 (11.8–201.2)	28.9 (6.0–116.3)	216.7 (36.4–673.5)	–	–

ANAE: anaerobic culture on selective media; CDI: Clostridium difficile infection; CIA: chromatographic immunoassay (two separate tests for GDH and toxins A/B); CLIA: chemiluminescent immunoassay (two separate tests for GDH and toxins A/B); GDH: glutamate dehydrogenase; LFIA: lateral flow immunoassay (simultaneous tests to detect GDH and toxins A/B; NAAT: nucleic acid amplification test; S: secondary care hospital; SC: specialised centre; SD: standard deviation; T: tertiary care hospital; TC: anaerobic culture on selective media followed by LFIA (Hospital B) or NAAT (Hospital L).

[a] Calculated using the Shannon index [16].

CC7 (7/2); CC8 (7/3); CC9 (5/2); CC10 (4/1); CC11 (4/2); CC12 (4/3); CC13 (4/1); CC14, 16 and 17 (3/1); CC15 and 18 (3/2); CC19, 20, 23 and 27 (2/1); CC21, 22, 24, 25 and 26 (2/2) (Figure 3).

MLVA showed an STRD ≥ 3 to ≤ 10 in 33 isolates and an STRD > 10 in three isolates (Figure 3).

The minimum spanning tree of ribotype 001 isolates revealed 14 clonal complexes of 141 isolates (76.6%) (Table 3). The clonal complexes, with the number of isolates/number of hospitals in which they were found shown in parentheses, were as follows: CC1 (67/7); CC2 (21/3); CC3 (11/1); CC4 (7/4); CC5, 6 (6/1); CC7 (5/1); CC8 (5/5); CC9 (3/2); CC10, 11, 12 and 13 (2/1); CC14 (2/2). MLVA showed an STRD ≥ 3 to ≤ 10 in 32 isolates, and an STRD > 10 in 15 isolates, including isolates from CC11 and CC13 (Figure 4).

Discussion

In 2008, three Czech tertiary care hospitals participated in the European *C. difficile* infection study (ECDIS) [1]. In 2012–13, 10 Czech hospitals (nine tertiary care, three of which had participated in the 2008 study, and one secondary care) took part in the European, multicentre, prospective, biannual, point-prevalence study

Table 2

MLVA characteristics of *Clostridium difficile* PCR ribotype 176 isolates (n = 225) from 17 of 18 hospitals participating in survey of incidence of *C. difficile* infection, Czech Republic, 2014

Hospital	Total number of isolates	Number of ribotype 176 isolates	Number of ribotype 176 isolates in clonal complexes	Clonal complex number/ number of ribotype 176 isolates in the clonal complex	Presence of 100% identical[a] ribotype 176 isolates within a hospital	Presence of 100% identical[a] ribotype 176 isolates between hospitals
A	63	7	6	16/3; 17/3	Yes	No
B	49	16	13	1/1; 2/3; 5/4; 9/1; 22/1; 23/2; 26/1	Yes	Yes (Hospitals L, I)
C	59	4	1	11/1	No	No
D	28	8	7	1/7	Yes	Yes (Hospital L)
E	50	17	16	1/15; 7/1	Yes	Yes (Hospital Q)
F	28	11	6	1/3; 8/2; 18/1	No	Yes (Hospital K)
G	15	3	3	1/1; 21/1; 24/1	No	Yes (Hospital L)
H	28	0	0	–	–	–
I	39	32	28	1/5; 2/11; 5/4; 10/4; 15/2; 19/2	Yes	Yes (Hospitals L, O, B)
J	45	2	1	22/1	No	No
K	17	9	5	4/1; 12/2; 18/2	Yes	Yes (Hospitals F, N)
L	167	50	46	1/13; 2/1; 3/19; 5/2; 8/3; 9/4; 21/1; 25/1; 24/1; 26/1	Yes	Yes (Hospitals B, D, G, I)
M	29	14	13	6/11; 20/2	Yes	No
N	36	26	23	4/9; 7/6; 8/2; 12/1; 13/4; 25/1	Yes	Yes (Hospital K)
O	38	13	12	1/5; 2/4; 4/1; 27/2	Yes	Yes (Hospital I)
P	17	3	2	1/1; 15/1	No	No
Q	14	5	4	1/1; 14/3	Yes	Yes (Hospital E)
R	52	5	4	11/3; 12/1	No	No
Total	**774**	**225**	**190**	–	**11 hospitals**	**11 hospitals**

MLVA: multilocus variable-number tandem repeat analysis.

[a] Sum of tandem repeat differences (STRD) = 0.

of CDI in hospitalised patients with diarrhoea (EUCLID) [2]. Our current study, which involved 18 hospitals distributed across the Czech Republic (including seven tertiary, 10 secondary and one specialised healthcare facility) reflects better the CDI epidemiological situation in the country. Of these 18 hospitals, eight (seven tertiary care and one secondary care) also participated in EUCLID.

In the Czech Republic, it is mandatory to report cases of CDI to EPI-DAT, the Czech reporting system for infectious diseases, but CDI is reported as 'other bacterial intestinal infections'. Colonisation by *C. difficile* is not mandatorily reportable. An increasing incidence of other bacterial intestinal infections was observed, from 26.4 per 100,000 habitants in 2005 to 64.3 per 100,000 habitants in 2014 [21]. As it is impossible to determine which of these infections are CDIs, however, CDI incidence data among hospitalised patients can only be derived from our study and the European studies mentioned above.

The results of our study showed a mean incidence of CDI per hospital of 6.1 cases per 10,000 patient bed-days and 37.8 cases per 10,000 admissions. Compared with incidence data for the Czech Republic in the 2008 European study [1], the incidence of CDI in the country has dramatically increased. Our findings are similar to those of EUCLID, which reported an incidence rate of 6.2 CDI cases per 10,000 patient bed-days in 2012–13 for the Czech Republic [2].

Our study also showed that the mean reported testing frequency was 39.5 tests per 10,000 patient bed-days, which is 1.7 times less than the mean testing frequency reported in EUCLID (65.8 tests per 10,000 patient bed-days) and almost three times less than the mean testing frequency reported for the United Kingdom (139 tests per 10,000 patient bed days) [2]. This indicates that CDI in the Czech Republic is most likely underdiagnosed and highlights the need for improvement of clinical awareness and laboratory algorithms (by adding a confirmatory test for GDH positive and toxin A/B-negative stool samples from patients with clinical symptoms of CDI).

It should be noted that considerable variation in CDI incidence was seen between the 18 participating hospitals. The highest incidence seen in Hospital K is probably due to the fact that this hospital also had

Table 3

MLVA characteristics of *Clostridium difficile* PCR ribotype 001 isolates (n = 184) from 14 of 18 hospitals participating in survey of incidence of *C. difficile* infection, Czech Republic, 2014

Hospital	Total number of isolates	Number of ribotype 001 isolates	Number of ribotype 001 isolates in clonal complexes	Clonal complex number/ number of ribotype 001 isolates in the clonal complex	Presence of 100% identical[a] ribotype 001 isolates within a hospital	Presence of 100% identical[a] ribotype 001 isolates between hospitals
A	63	38	32	1/19; 6/6; 7/5; 11/2	Yes	Yes (Hospitals C, E, H, R)
B	49	2	0	–	No	No
C	59	21	13	1/11; 2/1; 8/1	Yes	Yes (Hospitals A, E, M, R)
D	28	2	1	4/1	No	No
E	50	7	3	1/1; 4/1; 8/1	No	A, C
F	28	0	0	–	–	–
G	15	3	2	1/1; 8/1	No	Yes (Hospital R)
H	28	24	21	1/4; 3/11; 4/4; 8/1; 9/1	Yes	Yes (Hospital A)
I	39	0	0	–	–	–
J	45	24	21	2/19; 8/1; 14/1	Yes	No
K	17	0	0	–	–	–
L	167	9	4	2/1; 13/2; 14/1	Yes	No
M	29	8	5	1/3; 12/2	Yes	Yes (Hospitals C, R)
N	36	1	0	–	–	–
O	38	3	2	10/2	No	No
P	17	0	0	–	–	–
Q	14	1	1	4/1	No	No
R	52	41	36	1/28; 5/6; 9/2	Yes	Yes (Hospitals A, C, G, M)
Total	**774**	**184**	**141**	–	**7 hospitals**	**7 hospitals**

MLVA: multiocus variable-number tandem repeat analysis.

[a] Sum of tandem repeat differences (STRD) = 0.

the highest testing frequency, as there were sufficient local financial sources for extensive CDI testing. The high incidence in this hospital had a considerable impact on the SD of the mean incidence for all 18 hospitals. Despite the high incidence, the number of isolates submitted during the study was small. The sending of strains was voluntary and this hospital was unable to send a representative number of isolates.

All 18 participating hospitals used GDH testing, as a recommended screening step [22]. A total of 14 used a lateral flow immunoassay for a single GDH and toxin A/B test as the first step of their testing algorithm; the other four used these tests separately. The use of two separate tests is more economical because testing can be stopped when samples are GDH negative, as a GDH-negative result has a high predictive value for the absence of CDI [23].

Of the 18 hospitals, eight did not confirm toxin production in GDH-positive and toxin A/B-negative stool samples, although they performed anaerobic culture; thus their testing algorithm were suboptimal. Diagnostic uncertainty of diarrhoeal patients with a positive GDH test and negative toxin A/B tests because of a lack of a confirmatory test may have also contributed to the spread of CDI in the Czech Republic.

It is clear that CDI diagnostic testing in the Czech Republic is very variable. A web-questionnaire, completed by 61 laboratories in 2014 showed that 21% (n = 13) used only GDH and toxin A/B test, and 8% (n = 5) used toxin A/B test as a screening test [24].

Ribotyping of *C. difficile* isolates in our study revealed the presence of PCR ribotype 176 in 29% and PCR ribotype 001 in 24% of isolates. The frequent occurrence of PCR ribotype 176 simultaneously with PCR ribotype 027 was reported in Poland in 2008–13 [10,11]. In 2013, the first sporadic case of an imported infection caused by PCR ribotype 027 was found in the Czech Republic [25]. In the study presented here, we diagnosed two new CDI cases due to PCR ribotype 027 infection: one was a man in his 30s, the other a man in his 70s.

Whereas PCR ribotype 176 has been only reported from two countries (Czech Republic and Poland) [10,11,25],

which neighbour each other, PCR ribotype 001 has been problematic for a long time in many European countries [1,2,26]. It has dominated, as in Slovakia in 2012 [27], or has occurred together with PCR ribotype 027, as reported from Germany (Hesse region) in 2011–13 [28], the north-east of England in July 2009 to December 2010 [29] and Scotland in November 2007 to December 2009 [30]. It has also occurred together with other PCR ribotypes, such as 014/020 and 126/078, in a single-day study in Spain [31].

MLVA of the two predominant ribotypes identified in our study revealed close genetic relatedness between isolates of each ribotype. The occurrence of 100%-identical (STRD = 0) PCR ribotype 176 isolates in 11 hospitals and PCR ribotype 001 isolates in seven hospitals, suggests clonal clusters within and between healthcare facilities, probably due to ineffective hospital infection control measures and transfer of patients between healthcare facilities who were in fact CDI cases but had not been diagnosed. This is supported by the observation of clonal complexes in tertiary and secondary hospitals in the same region. The question remains as to which specific molecular characteristics of PCR ribotypes 176 and 001 allow them to spread rapidly within healthcare facilities in contrast to the other less frequent PCR ribotypes identified in the study.

Antibiotic susceptibility testing of *C. difficile* isolates was not performed in this study but multiresistance of PCR ribotype 176 isolates [32,33], as well as PCR ribotype 001 isolates, has been reported [26,34]. The results of a recently published European study on antibiotic resistance among prevalent *C. difficile* ribotypes showed the Czech Republic as a country with a high cumulative resistance score (4–5), calculated based on susceptibility to nine antimicrobials tested [26].

An important limitation of our study is the lack of clinical patient data. The Czech national reference centre for healthcare-associated infections is currently organising the implementation of CDI surveillance based on the recent CDI surveillance protocol from the European Centre for Disease Prevention and Control [35]. The first national CDI incidence data, including clinical data on CDI patients and data on antibiotic susceptibility to metronidazole, vancomycin and moxifloxacin of *C. difficile* isolates, should be available in 2016 (CDI surveillance started in April 2016 in the Czech Republic).

Conclusion

The results of our study showed an unfavourable CDI epidemiological situation in the Czech Republic in 2014 caused by the occurrence of epidemic PCR ribotypes 176 and 001. The absence of national surveillance at that time, the low frequency of testing and variability in testing algorithms probably contributed to the spread of these PCR ribotypes.

A Czech standardised CDI testing protocol and the implementation of CDI surveillance in a large number of hospitals is urgently needed for monitoring, management and reduction of these infections in the Czech Republic.

Members of the Czech Clostridium difficile study group (in no particular order)

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Conflict of interest

None declared.

Authors' contributions

ON, PD, MK, JM designed the study. ON, JM supervised CDI diagnostics. MK performed molecular characterisation of C. difficile isolates. Study group members provided C. difficile isolates, data on diagnostics algorithm and annual incidence and testing data. MK, ON, EK analysed the collected data. MK wrote the first draft of the manuscript. EK, ON, PD critically revised the subsequent drafts of the manuscript. All

authors approved the final version of the manuscript and agreed with its submission.

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