SURVEILLANCE AND OUTBREAK REPORT

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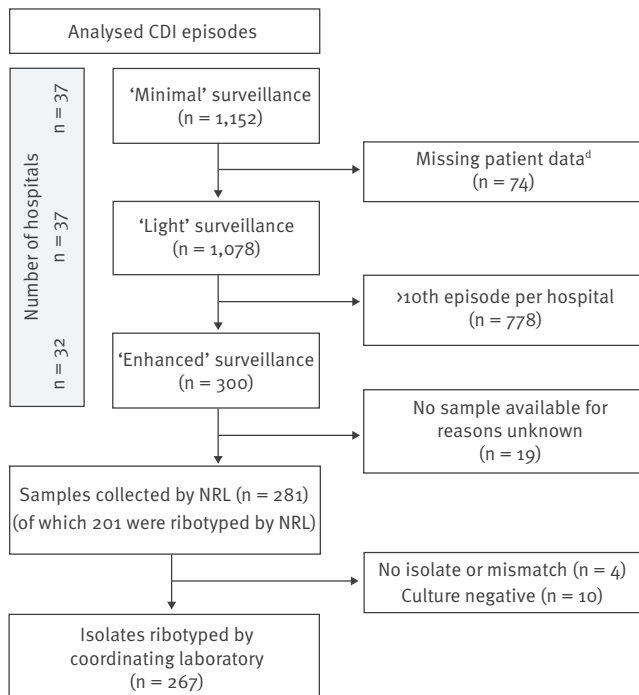 10,000 hospital discharges, respectively. Enhanced surveillance was tested and was successful in 32 of the hospitals, showing that *C. difficile* PCR ribotype 027 was predominant (30% (79/267)). This study showed that standardised multicountry surveillance, with the option of integrating clinical and 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

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]

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┌──────────────────────────────────┐
│       Analysed CDI episodes      │
└──────────────────────────────────┘

            ┌──────────────────────────┐
   n=37     │  'Minimal' surveillance  │
            │       (n = 1,152)        │────────►  ┌──────────────────────────┐
            └──────────────────────────┘           │   Missing patient data[d] │
   n=37                                             │        (n = 74)          │
            ┌──────────────────────────┐           └──────────────────────────┘
            │   'Light' surveillance   │
            │       (n = 1,078)        │────────►  ┌──────────────────────────┐
            └──────────────────────────┘           │  ›10th episode per hospital │
   n=32                                             │        (n = 778)         │
            ┌──────────────────────────┐           └──────────────────────────┘
            │ 'Enhanced' surveillance  │
            │        (n = 300)         │────────►  ┌──────────────────────────┐
            └──────────────────────────┘           │  No sample available for │
                                                   │    reasons unknown       │
┌──────────────────────────────────┐               │        (n = 19)          │
│ Samples collected by NRL (n = 281)│               └──────────────────────────┘
│ (of which 201 were ribotyped by   │
│          NRL)                     │────────►  ┌──────────────────────────┐
└──────────────────────────────────┘           │ No isolate or mismatch (n = 4)│
                                                │  Culture negative (n = 10)   │
┌──────────────────────────────────┐           └──────────────────────────┘
│       Isolates ribotyped by      │
│     coordinating laboratory      │
│          (n = 267)               │
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Number of hospitals

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

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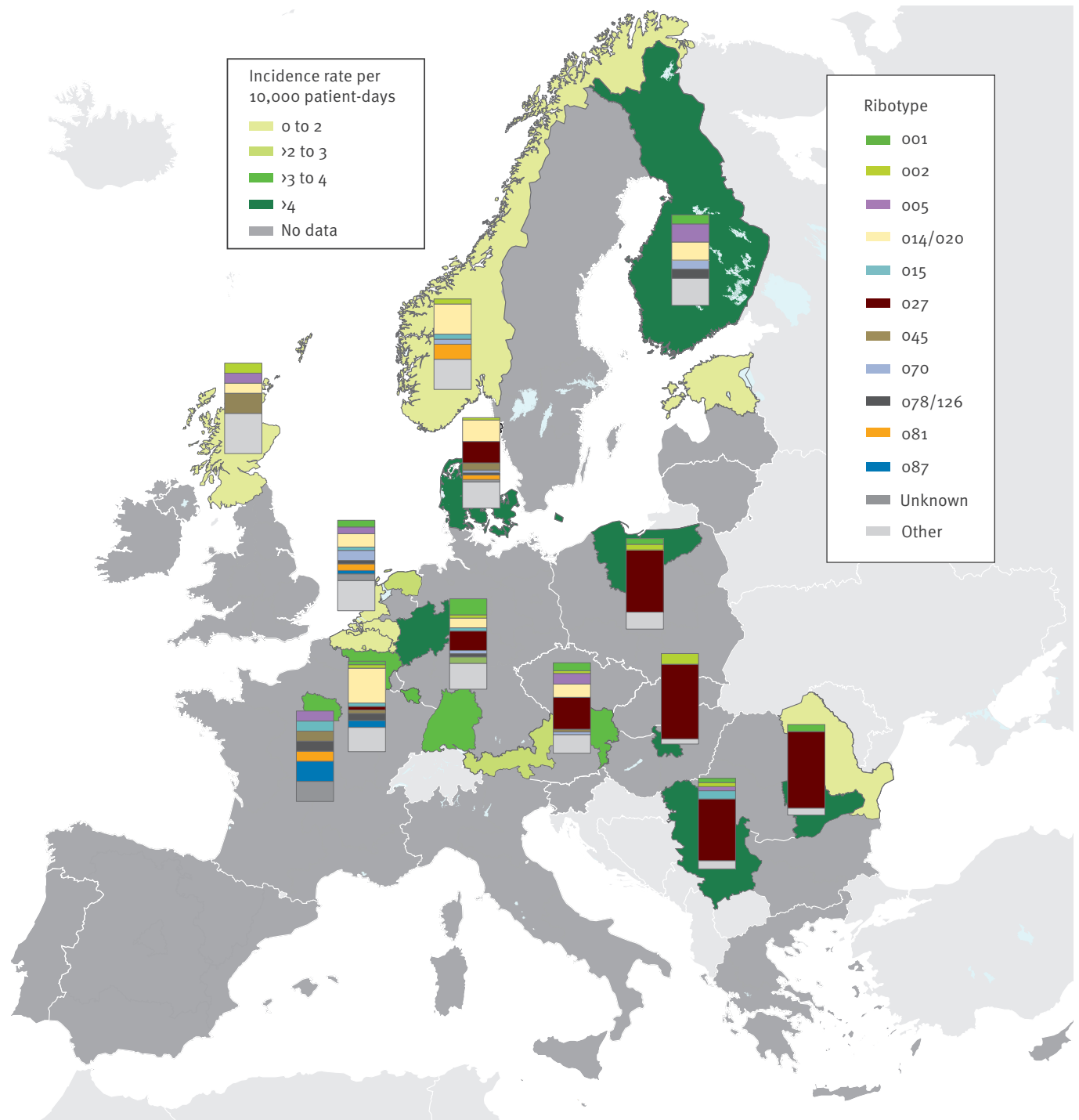
CDI: Clostridium difficile infection; NUTS: nomenclature of territorial units for statistics.

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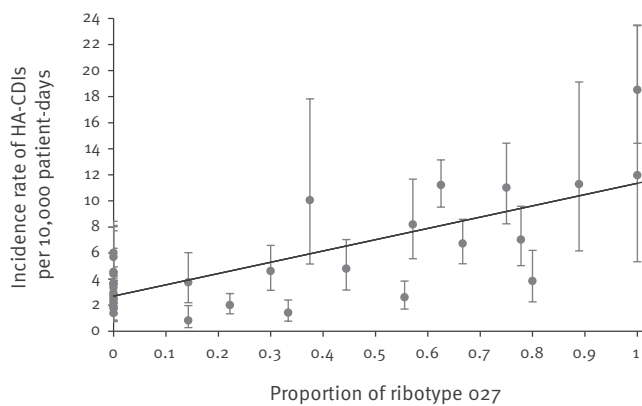
CA: community-associated; CDI: Clostridium difficile infection; CI: confidence interval; HA: healthcare-associated.

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

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 disproportionly 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.

Conflict of interest

Authors' contributions

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