Clostridium difficile infection : epidemiology, complications and recurrences

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Chapter 4

Clostridium difficile infection in Europe: a hospital-based survey

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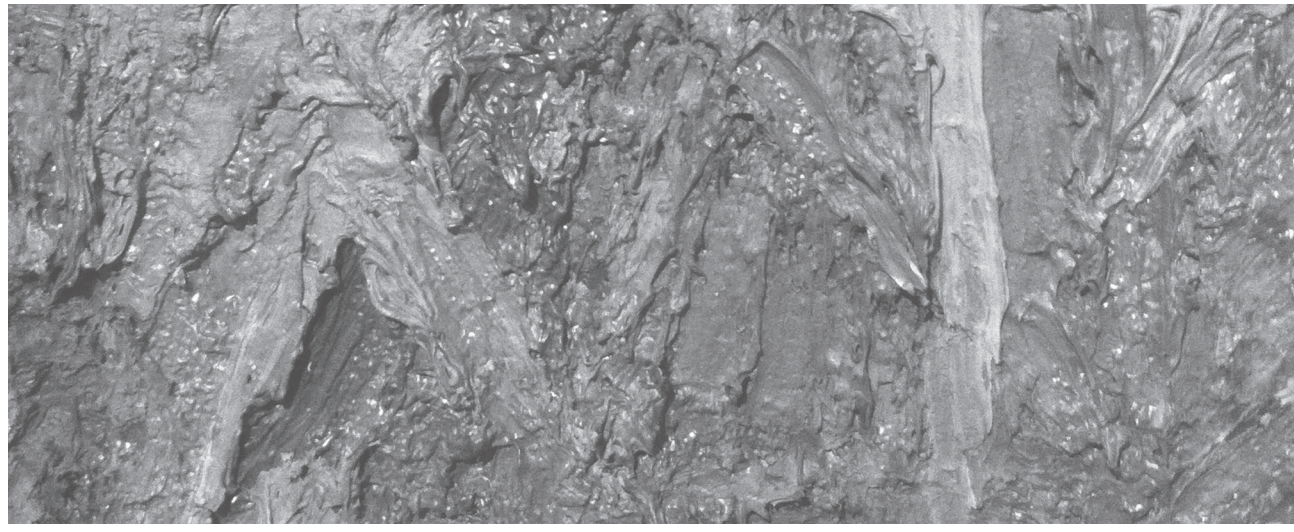

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Summary

Background

Little is known about the extent of *Clostridium difficile* infection in Europe. Our aim was to obtain a more complete overview of *C. difficile* infection in Europe and build capacity for diagnosis and surveillance.

Methods

We set up a network of 106 laboratories in 34 European countries. In November, 2008, one to six hospitals per country, relative to population size, tested stool samples of patients with suspected *C. difficile* infection or diarrhoea that developed 3 or more days after hospital admission. A case was defined when, subsequently, toxins were identified in stool samples. Detailed clinical data and stool isolates were collected for the first ten cases per hospital. After 3 months, clinical data were followed up.

Findings

The incidence of *C. difficile* infection varied across hospitals (weighted mean 4.1 per 10,000 patient-days per hospital, range 0.0-36.3). Detailed information was obtained for 509 patients. For 389 of these patients, isolates were available for characterisation. 65 different PCR ribotypes were identified, of which 014/020 (61 patients [16%]), 001 (37 [9%]), and 078 (31 [8%]) were the most prevalent. The prevalence of PCR-ribotype 027 was 5%. Most patients had a previously identified risk profile of old age, comorbidity, and recent antibiotic use. At follow up, 101 (22%) of 455 patients had died, and *C. difficile* infection played a part in 40 (40%) of deaths. After adjustment for potential confounders, an age of 65 years or older (adjusted odds ratio 3.26, 95% CI 1.08-9.78; p=0.026), and infection by PCR-ribotypes 018 (6.19, 1.28-29.81; p=0.023) and 056 (13.01; 1.14-148.26; p=0.039) were significantly associated with complicated disease outcome.

Interpretation

PCR ribotypes other than 027 are prevalent in European hospitals. The data emphasise the importance of multicountry surveillance to detect and control *C. difficile* infection in Europe.

Funding

European Centre for Disease Prevention and Control.

Introduction

Clostridium difficile infection is prevalent in health-care facilities throughout the developed world, but also presents as large outbreaks. Less often, it is acquired in the community from an unknown source. It characteristically occurs in elderly patients with comorbidity in whom the intestinal flora has been disrupted by previous use of antibiotics.[1,2] Since early 2003, increasing rates of *C. difficile* infection have been reported in Canada and the USA, with a larger proportion of severe and recurrent cases occurring in these countries than previously reported. The raised incidence and virulence of such infection have partly been explained by the spread of fluoroquinolone-resistant strains belonging to the PCR-ribotype 027.[3-5] In addition to the usual toxins A and B, these fluoroquinolone-resistant strains produce a binary toxin, with a hitherto uncertain pathogenic significance.[1-6] In Europe, PCR-ribotype 027 was first reported in 2005 in England and shortly thereafter in the Netherlands.[7,8] Subsequently, epidemics of *C. difficile* infection caused by PCR-ribotype 027 have been recognised in hospitals in many European countries.[9]

The attention given to this infection, diagnostic procedures in hospitals, presence and methodology of national surveillance, and availability of typing vary widely across Europe, which hampers comparisons between countries.[9,10] We did this study to obtain a more complete overview of the situation in Europe and build capacity for diagnosis and surveillance of *C. difficile* infection both nationally and Europe-wide.

Methods

Study design and patients

With support from the European Centre for Disease Prevention and Control, we appointed national coordinators for 34 European countries (including 27 member states, three candidate states, and four European-Free-Trade-Association countries) who selected hospitals in each country, relative to the country's population size. No randomisation was used for this selection. The aim was to include one hospital for countries with fewer than two million inhabitants, three for those with between two and 20 million inhabitants, and five for those with more than 20 million inhabitants, with a balance between academic and non-academic institutions. A study protocol noting all procedures was distributed to national coordinators and coordinators in all hospitals. Hospitals and laboratories completed a web-based questionnaire (Appendix) with epidemiological data, including numbers of patient-days, admissions, and stool samples tested for *C. difficile* infection in November, 2008, and technical data such as assays and culture methods used.

Procedures

Hospitals were asked to test for *C. difficile* infection in outpatients and inpatients suspected of having the infection by their treating physician and all inpatients who developed diarrhoea 3 days or more after admission. Clinical grounds on which to suspect recurrence were left to the attending physicians' judgment, who could use the definition of *C. difficile* infection according to the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) treatment guidance for *C. difficile* infection.[11] Only patients aged 2 years or older were included in the study. Patients with suspected *C. difficile* infection and diarrhoea, whose stool samples were positive for toxin A, B, or both (EIA, cytotoxicity test, or PCR) or revealed the presence of toxin-producing *C. difficile* were defined as having *C. difficile* infection.

A web-based questionnaire (Appendix) was used to gather additional information about demography, clinical data, and risk factors associated with the infection in the first patients to be diagnosed, with a maximum of ten patients included per participating hospital. If patients had episodes of *C. difficile* infection in the previous8 weeks, they were reported as having recurrent disease at inclusion. Stool samples from the first ten patients were cultured for *C. difficile* according to local protocols, and the isolates were sent to a central laboratory (Leiden University Medical Centre, Leiden, Netherlands) for further characterisation.

3 months after diagnosis, follow-up clinical data were obtained as part of the web-based questionnaire, including overall mortality, mortality attributable to *C. difficile* infection, colectomy, intensive-care-unit (ICU) admission, and recurrences during follow-up. Clinical grounds on which to suspect recurrence were left to the attending physicians' judgment, who could use the definition of recurrence according to the ESCMID treatment guidance for *C. difficile* infection.[11] All patients suspected of recurrence, who had toxin-positive-stool samples, were reported as having recurrence. No attempt was made to differentiate between relapses and reinfections. Identification of *C. difficile* was confirmed by an in-house PCR test for the glutamate dehydrogenase gene specific to *C. difficile*.[12] Isolates were further characterised by PCR ribotyping.[13] Since PCR-ribotypes 014 and 020 are nearly identical and differ only by one band on a specific agarose-gel electrophoresis, the types were reported together as ribotype 014/020. The presence of toxin A, toxin B, and binary toxin genes were investigated with standardised PCRs.[14,15] Isolates that were difficult to type were sent to the Anaerobe Reference Laboratory in Cardiff, UK, for further characterisation by the Cardiff PCR-ribotyping library, which currently consists of more than 300 ribotypes.[16] These isolates, and isolates of PCR ribotypes for which the toxinotype was unknown, were sent to the Institute of Public Health in Maribor, Slovenia, for toxinotyping.[17] No attempt was made to identify more than one causative ribotype, because infection by *C. difficile* resulting from more than one ribotype is thought to be rare.

We adhered to the epidemiological recommendations as defined by the ad hoc *C. difficile* surveillance working group.[1,18] Briefly, *C. difficile* infection is divided into health-care-associated cases (i.e., occurring in a hospital or nursing home after 48 h of admission or within 4 weeks after discharge from such a facility), community-associated cases (i.e., occurring in the community, provided that the patient had not been admitted to a health-care facility in the previous 12 weeks), and an indeterminate group for infections occurring between 4 and 12 weeks after discharge from a health-care facility. Furthermore, complicated disease was defined as *C. difficile* infection that contributed to or caused ICU admission or death, or led to colectomy. Severe comorbidity was defined as having a chronic-health points score over 0, as defined by the Acute Physiology, Age, Chronic Health Evaluation (APACHE) II score.[19] Quinolones were classified as old quinolones (nalidixic acid, norfloxacin, ofloxacin, ciprofloxacin) and new quinolones (levofloxacin, moxifloxacin, gatifloxacin).

Statistical analysis

For all hospitals, incidence rates of health-care-associated *C. difficile* infection were obtained by dividing the number of health-care-associated occurrences in November, 2008, (extrapolated by multiplication of the proportion of healthcare-associated infection in the questionnaires with all cases recorded in November, 2008) by the number of patient-days in November, 2008. Health-care-associated *C. difficile* infection incidence rates were also calculated with the total number of admissions as the denominator. Weighted mean incidence rates per hospital were calculated for each country from the incidence rates of all hospitals in that country, using the number of patient-days and the number of admissions per hospital as a weighting factor.

The associations of patient and pathogen characteristics with two outcome measures (complicated infections or recurrence within the 3-month follow up) were investigated. Since patients were nested within hospitals and might be exposed to common characteristics of their hospitals that could be important determinants of outcome, we could not assume independence of observations. Therefore, we chose a two-level multilevel-regression method, which takes into account within-group (hospital) and between-group relations, and allows for integration of hospital and patient variables. Since the outcome (complicated infection or recurrence) was binary, we used the logistic form of the multilevel-regression model. An odds ratio with a 95% CI was calculated for all associations between a patient or pathogen characteristic and an outcome—i.e., complicated infection or recurrence. Cases in which non-toxigenic strains were cultured were classified as culture negative, since these strains were not thought to be the cause of symptoms. Many of the associations reported in the analysis could be subject to confounding. For example, an association between the acquisition of *C. difficile* infection in a health-care facility (as opposed to

the community) and a complicated outcome might be confounded by age. To adjust the odds ratios for such potential confounders, we did a multivariate analysis for a selection of variables, again using a two-level logistic-regression model. As potential confounders, we selected variables for which a role as a confounder was biologically plausible and that were correlated to outcome with an alpha level less than 0.2, since significance-selection strategies to select for possible confounders do best at this level.[20] We tested whether confounders were highly collinear (variance inflation factor >10), in which case only one of them would be introduced as a covariate in multivariate analysis. Generally, statistical significance was declared for p values less than 0.05. Data were analysed with Stata 10.1.

Role of funding source

The study was funded by the European Centre for Disease Prevention and Control (ECDC) through a specific service contract (ECD.894) to the Centre for Infectious Disease Control Netherlands, National Institute for Public Health and the Environment, Bilthoven, Netherlands. The decision to submit for publication was taken by the study coordinator in the Netherlands. ECDC provided support on the study design, suggested national coordinators, and provided comments on the analysis and the final report.

Results

In total, 97 hospitals provided patients or epidemiological data, or both. Because some hospitals were unable to supply denominator data, we could not calculate incidences for all hospitals (table 1). Most hospitals were large, as judged by the number of patient-days and admissions (median number of admissions per month 2,645; IQR 1,808-4,257); 62 hospitals (67%) were academic hospitals. The estimated incidence of health-care-associated infection varied widely between hospitals. We calculated the proportion of health-care-associated *C. difficile* infection by the sum of health-care-associated and community-associated infections (table 1).

We tested associations between high-incidence hospitals (>10 per 10,000 patient-days) and antibiotics used by the patients in the month preceding inclusion. Use of aminopenicillins (odds ratio [OR] 2.70, 95% CI 1.17-6.22), first-generation cephalosporins (6.98, 1.83-26.62), or second-generation cephalosporins (2.40, 1.28-4.50) was significantly associated with high-incidence hospitals.

395 isolates from 73 hospitals in 26 countries were available for detailed characterisation. 65 different PCR ribotypes were identified (figure), including six new PCR ribotypes: 228, 229, 230, 231, 232, and 234. The most common PCR ribotypes were 014 and 020 (found in 19 countries), 001 (in 13 countries), and 078 (in 18 countries); PCR ribotype 027 ranked sixth (in six countries; table 2). Some commonly encountered PCR ribotypes were identified in a few countries and their distribution suggested regional spread (figure). Among these were PCR ribotype 106, which was reported in the UK (13 isolates), Ireland (five), and Spain (two), and PCR ribotype 018, which was recorded in Italy (19), Spain (two), Austria (one), and Slovenia (one). 12 different toxinotypes were identified. Of these, toxinotype 0 was most prevalent, representing 248 (65%) of 383 isolates; toxinotype III was identified predominantly in PCR-ribotype 027 strains (19 isolates) and only in five isolates belonging to rare PCR ribotypes (075, 099, 176, and 208); toxinotype IV predominantly in PCR-ribotype 023; and toxinotype V in PCR ribotypes 078 (30 isolates) and 126 (12); toxinotype XII fully correlated with PCR-ribotype 056. 13 (3%) isolates were *C. difficile*-toxin-A negative and *C. difficile*-toxin-B positive. 11 of these isolates belonged to PCR ribotype 017 and one each to the newly identified PCR ribotypes 232 and 234. Six (2%) isolates were non-toxigenic and were not included in further analyses.

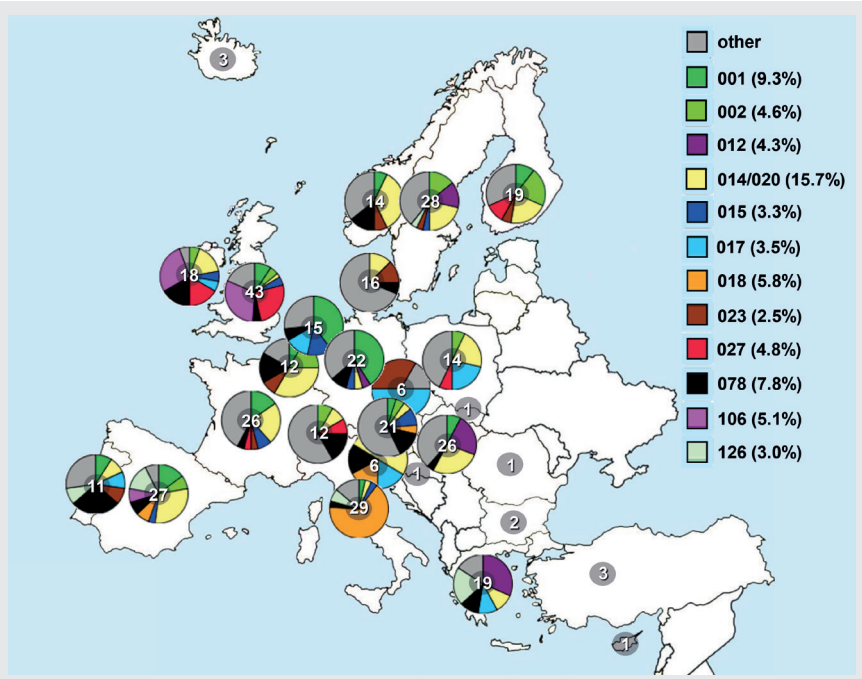

Figure Geographical distribution of *Clostridium difficile* PCR ribotypes in European countries with more than five typable isolates, November 2008.

Pie charts show proportion of most frequent PCR-ribotypes per country. The number in the centre of pie charts is the number of typed isolates in the country.

Table 1 Summary of *Clostridium difficile* infection in countries and hospitals.

	Number of toxin-positive cases/number of patients tested	Number of patients tested per 10,000 patient-days	Number of participating hospitals*	Weighted mean health-care-associated *C. difficile* infection incidence rate per hospital (minimum to maximum range) †		Percentage of health-care-associated *C. difficile* infection cases in health-care-associated and community-associated *C. difficile* infections	Number of complicated cases/ number of cases with available data (%)	Toxin tests used (number of hospitals)
				per 10,000 patient-days	per 10,000 admissions			
Austria	53/ 330 (16%)	52	3	7.5 (4.3 - 10.9)	36 (20 - 46)	92%	4/ 26 (15%)	A+B (2); A+B and Cu (1)
Belgium	16/ 283 (6%)	55	3	2.8 (0.0 - 6.2)	19 (0 - 39)	91%	0/ 11 (0%)	A+B (1); Cy and A+B (1); A (1)
Bulgaria	2/ 9 (22%)	3	3	0.6 (0.0 - 2.1)	3 (0 - 10)	100%	1/ 1 (100%)	A+B (3)
Croatia	22/ 197 (11%)	41	3 (2)	0.7 (0.5 - 2.1)	6 (4 - 20)	18%	1/ 14 (7%)	A+B (2)
Cyprus	1/ 28 (4%)	34	1	1.2	5	100%	0/ 1 (0%)	A+B (1)
Czech Republic	10/ 152 (7%)	17	3	1.1 (0.0 -1.3)	7 (0 - 9)	100%	2/ 7 (29%)	A+B (3)
Denmark	28/ 330 (8%)	74	3	5.5 (4.4 - 9.6)	18 (10 - 25)	88%	1/ 19 (5%)	A+B (1); Cu (2)
Finland	52/ 351 (15%)	141	3	19.1 (8.7 - 28.5)	80 (30 - 132)	91%	2/ 22 (9%)	A+B and Cu (1); Cu (1); A&B (1)
France	37/ 626 (6%)	42	5 (4)	2.1 (1.0 - 3.1)	15 (6 - 27)	84%	4/ 34 (12%)	A+B (2); Cu (1); Cy (1)
Germany	93/ 602 (15%)	72	6 (5)	7.4 (2.9 - 16.4)	60 (25 - 276)	91%	2/ 24 (8%)	A+B (3); Cu (1); Cy (1)
Greece	21/ 288 (9%)	60	3	3.7 (1.3 - 4.9)	29 (9 - 44)	84%	0/ 17 (0%)	A+B (3)
Hungary	22/ 333 (7%)	38	3	2.0 (0.4 - 3.9)	9 (1 - 23)	68%	1/ 25 (4%)	A+B (3)
Iceland	6/0	..	1	..	..	100%	0/ 6 (0%)	..
Ireland	38/ 493 (8%)	94	3	7.3 (6.5 - 7.9)	63 (39 - 92)	100%	5/ 21 (24%)	A+B (3)
Italy	57/ 533 (11%)	39	5	3.6 (0.4 - 5.8)	22 (2 - 61)	85%	5/ 18 (28%)	A+B (2), GluD and A+B (1); Cy (1)
Latvia	13/ 64 (20%)	10	3	1.9 (0.0 - 2.8)	13 (0 - 20)	91%	0/ 13 (0%)	A (2); A+B (1)
Luxembourg	0/ 28 (0%)	49	1	0.0	0	NA	0	A+B
Netherlands	18/ 309 (6%)	69	3	4.0 (2.3 - 8.5)	23 (13 - 43)	100%	1/ 15 (9%)	A+B (2); Cy (1)
Norway	37/ 241 (15%)	50	3	7.6 (0.4 - 16.5)	56 (3 - 229)	100%	1/ 16 (6%)	A+B (3)
Poland	102/ 263 (39%)	45	3	12.5 (3.8 - 36.3)	76 (29 - 189)	79%	1/ 11 (9%)	A+B (2); Cu (1)
Portugal	14/ 158 (9%)	45	3 (2)	2.6 (1.9 - 8.2)	13 (13 - 14)	86%	0/ 10 (0%)	A+B (3)
Romania	1/ 11 (9%)	3	5 (1)	0.3	2	100%	0/ 1 (0%)	A+B (2)
Slovakia	10/ 91 (11%)	16	3 (2)	1.4 (0.0 - 2.1)	11 (0 - 15)	71%	0/ 5 (0%)	A (1); Cu (1)
Slovenia	24/ 123 (20%)	17	3 (2)	2.8 (1.5 - 3.2)	19 (10 - 23)	67%	1/ 10 (10%)	A+B (2)
Spain	46/ 485 (9%)	45	5	4.3 (0.0 - 16.7)	30 (0 - 47)	100%	5/ 28 (18%)	A+B (2); Cu (1); A+B and Cy and Cu (1); A+B and Cu (1)
Sweden	69/ 430 (16%)	74	3	9.8 (6.3 - 15.7)	50 (28 - 71)	86%	2/ 30 (7%)	A+B (2); Cy (1)
Switzerland	16/ 150 (11%)	45	3	4.8 (0.0 - 7.5)	50 (0 - 84)	100%	0/ 12 (0%)	A+B (2); Cu (1)
Turkey	4/ 105 (4%)	4	5	0.0 (0.0 - 0.6)	0 (0 - 4)	20%	0/ 4 (0%)	A+B (3); A (1)
United Kingdom	164/ 1,695 (10%)	115	6	10.6 (6.7 - 30.3)	50 (44 - 135)	92%	5/ 40 (13%)	A+B (3); Cy (3)
Total	NA	NA	97 (87)	4.1 (0.0 - 36.3)	23 (0 - 276)	NA	44/ 442 (10%)	NA

A+B=enzyme immunoassay for *C. difficile* toxin A and B. A=enzyme immunoassay for *C. difficile* toxin A only. Cu=toxigenic culture. Cy=cytotoxicity test. GluD=enzyme immunoassay for *C. difficile*-specific glutamate dehydrogenase. NA=not applicable. ..=data not available. *Number of hospitals on which incidence data are based is shown in parentheses. The remaining hospitals did not provide denominator data. †Weight factor for weighted-mean incidence per 10,000 patient-days=number of patient-days; weight factor for weighted-mean incidence per 10,000 admissions=number of admissions. The UK and Germany were each granted one extra hospital. In Poland, three hospitals rather than five were recruited. No hospitals were recruited in Lithuania, and one was recruited in Malta. From Estonia, Liechtenstein, and the Former Yugoslav Republic of Macedonia no data or isolates were received.

Table 2 Characteristics of patients with *Clostridium difficile* infection for whom questionnaires were completed.

	n/ N (%)
Epidemiological characteristics	
Female	287/ 509 (56%)
Age ≥65 years*	319/ 509 (63%)
Epidemiological association	
Health-care associated	408/ 506 (80%)
Community associated	70/ 506 (14%)
Indeterminate association	28/ 506 (6%)
Explicit request to test for infection	441/ 507 (87%)
Use of an antibiotic not directed at *C. difficile* infection	
Any antibiotic not directed at *C. difficile* infection	366/ 463 (79%)
Aminopenicillin	28/ 463 (6%)
Aminopenicillin - β-lactamase inhibitor combination	86/ 463 (19%)
Antipseudomonal penicillin - β-lactamase inhibitor combination	38/ 463 (8%)
Second-generation cephalosporin	60/ 463 (13%)
Ceftazidime	78/ 463 (17%)
Any cephalosporin	155/ 463 (34%)
Carbapenem	41/ 463 (9%)
Aminoglycoside	27/ 463 (6%)
Old quinolone	80/ 463 (17%)
New quinolone	29/ 463 (6%)
Any quinolone	104/ 463 (23%)
Intravenous glycopeptide	33/ 463 (7%)
Lincosamide	28/ 463 (6%)
Macrolide	27/ 463 (6%)
Co-trimoxazole	25/ 463 (5%)
Use of any antibiotic not directed at *C. difficile* infection during previous 3 months	426/ 463 (92%)
Comorbidity	
Severe comorbidity (APACHE II CHP >0)	204/ 468 (44%)
Liver cirrhosis (APACHE II)	21/ 488 (4%)
Heart disease (APACHE II)	47/ 484 (10%)
Pulmonary disease (APACHE II)	54/ 480 (11%)
Chronic dialysis (APACHE II)	30/ 496 (6%)
Immunocompromised status (APACHE II)	106/ 488 (22%)
Treatment for inflammatory bowel disease	21/ 492 (4%)
Episodes of infection in previous 8 weeks	68/ 431 (16%)
Disease characteristics	
Outpatient	56/ 509 (11%)
Duration of diarrhoea	
<1 week	334/ 461 (73%)
1 to 3 weeks	92/ 461 (20%)
>3 weeks	35/ 461 (8%)

Table 2 Continued.

	n/ N (%)
Disease characteristics	
Diarrhoea mixed with blood at any moment in previous week	48/ 416 (12%)
Fever (temperature >38.5ºC)	167/ 446 (37%)
Ileus at any moment in previous week	20/ 509 (4%)
Last leukocyte count in previous week ≥15 × 10^9/L†	122/ 428 (29%)
Serum creatinine rise >50% compared to baseline before onset of symptoms	31/ 395 (8%)
Sigmoidoscopy or colonoscopy‡	
Pseudomembranes	7/ 29 (24%)
Ulceration	13/ 29 (45%)
Imaging‡	
Colonic wall thickening on CT	26/ 63 (41%)
Pericolonic fat stranding on CT	7/ 63 (11%)
Bowel distension on plain abdominal radiograph or CT	27/ 117 (23%)
Microbiological characteristics	
Most frequent PCR ribotypes among toxigenic isolates	
014/020	61/ 389 (16%)
001	37/ 389 (10%)
078	31/ 389 (8%)
018	23/ 389 (6%)
106	20/ 389 (5%)
027	19/ 389 (5%)
002	18/ 389 (5%)
012	17/ 389 (4%)
017	14/ 389 (4%)
015	13/ 389 (3%)
126	12/ 389 (3%)
023	10/ 389 (3%)
046	8/ 389 (2%)
003	7/ 389 (2%)
011	6/ 389 (2%)
053	6/ 389 (2%)
056	6/ 389 (2%)
Presence of either or both binary toxin genes in toxigenic isolates	90/ 389 (23%)
Toxin A negative, toxin B positive strains in toxigenic isolates	13/ 389 (3%)

All time periods mentioned are related to the time of collection of the stool sample. Only antibiotics that were administered to more than 5% of patients are given. APACHE II=acute physiology, age, chronic health evaluation version two. CHP=chronic health points. N=total number of patients for whom information was available.
*Median 71 (IQR 56 - 81). †Leucocyte count distribution ´10^9 per L (11; 11 - 15). ‡Data apply to current episode of *C.. difficile* infection. If several procedures were done during an episode, only the first was considered. §Two patients were treated for inflammatory bowel disease.

Most cases were health-care associated or community associated, leaving 6% of indeterminate association (table 2). Most patients fitted the previously established risk profile, with almost two-thirds aged 65 years or more, about two-fifths having severe comorbidity, and almost all having received antibiotics during the 3 months before their infection, most commonly cephalosporins, quinolones, and amino-penicillin - β-lactamase-inhibitor combinations (table 2). 68 (16%) of 431 patients had recurrent *C. difficile* at inclusion.

Data after 3-months' follow-up were obtained for about 90% of patients (table 3). An exact number cannot be provided, since follow-up was incomplete for some patients and therefore the number of patients with follow-up data differs for each variable. Of the 101 patients who had died, 40 (40%) of 101 deaths were judged to be related to *C. difficile* infection.

All seven patients who died from *C. difficile* infection as a main cause were aged 75 years or older and their infection was health-care associated. Six of them had severe comorbidity (four had pulmonary disease, three were immunocompromised, and two had heart disease). Two of these patients had a recurrent episode of infection at presentation. Two had leukocyte counts of 30 ´ 10^9 per L or greater and two of 4 ´ 10^9 per L or less. The strains causing these infections belonged to PCR-ribotypes 015, 018, 027 (two patients), and 056. No isolate could be obtained for two patients.

An age of 65 years or older, severe pulmonary comorbidity, previous use of a new quinolone, and infection by PCR-ribotypes 027, 015, and 018 were significant risk factors for complicated infections in univariate analysis (table 4). Patients with this comorbidity were distributed evenly among all hospitals. No disease characteristic—such as duration of diarrhoea, presence of fever, or leukocyte count—was significantly associated with complicated infection nor was the presence of binary toxin. After correction for potential confounders, an age of 65 years or older and infection by PCR-ribotypes 018 and 056 were significantly associated with complicated infection. These PCR ribotypes were binary-toxin negative and belonged to toxinotype 0 (type 018) and XII (type 056). The seven complicated cases caused by PCR-ribotype 018 occurred in four different hospitals in two countries, and the two complicated cases caused by PCR-ribotype 056 occurred in two hospitals in two countries.

An age of 65 years or older, previous use of ceftazidime, and recent episodes of *C. difficile* infection were significantly associated with recurrences during follow-up in univariate analysis (table 5). After correction for potential confounders, previous use of ceftazidime and recent episodes of infection were significantly associated with recurrence.

Since differences between patients with follow-up information and those without were possible, the characteristics of patients with available follow-up information about *C. difficile* infection complications (n=442) were compared with patients for whom this information was not available (n=67). Patients without this information were more likely to be outpatients at the time of presentation (OR 1.97, 95% CI 0.98 - 3.97), to have community-associated infection (2.59, 1.39 - 4.84), and be infected by PCR ribotype 018 (3.24, 1.20 - 8.73) or PCR ribotype 106 (3.96, 1.44 - 10.95); they were less likely to be aged 65 years or older (0.61, CI 0.36 - 1.02) and to have severe comorbidity (0.56, 0.31 - 1.01), especially pulmonary disease (0.26, 0.06 - 1.10). A separate analysis in which non-complicated *C. difficile* infection was assumed for patients with missing information resulted in closely similar values for the association of PCR-ribotype 018 with complicated infection (5.65; 1.63 - 19.57).

Table 3 Treatment and outcome (3-month follow up) characteristics of patients with *Clostridium difficile* infection.

	n/ N (%)
Initial episode treated with	
Oral metronidazole	341/ 477 (71%)
Intravenous metronidazole	50/ 472 (11%)
Oral vancomycin	89/ 483 (18%)
Intracolonic vancomycin	1/ 473 (0.2%)
ICU admissions	31/ 459 (7%)
CDI contributive	6/ 459 (1%)
CDI primary cause	1/ 459 (0.2%)
Colectomy for CDI	3/ 460 (0.7%)
Death	101/ 455 (22%)
CDI contributive	33/ 455 (7%)
CDI primary cause	7/ 455 (2%)
Complicated CDI	44/ 442 (10%)
Recurrent CDI*	86/ 484 (18%)
Both complicated and recurrent CDI	10/ 440 (2%)

Of 491 (96%) of 509 patients, complete or partial follow-up information was available. n=characteristics of patients with *Clostridium difficile* for whom questionnaires were completed. N=total number studied. ICU=intensive care unit. CDI=*C. difficile* infection. *Number of recurrences during follow-up in those patients who had recurrences: median 1; 1 - 3.

Because death or colectomy could have precluded a patient from having a recurrence, a separate analysis was done for risk factors for recurrence in only those patients who did not die or undergo a colectomy. Results of the univariate analysis mirrored the analysis for the whole group, except that previous use of intravenous glycopeptides and chronic dialysis were significantly associated with recurrence (3.28, 1.12 - 13.78 and 2.87, 1.02 - 8.14, respectively).

Different cutoff values for the continuous variables age and leukocyte count, as assessed by receiver operator characteristics, did not lead to improved performance in the prediction of complicated *C. difficile* infection.

Table 4 Determinants of complicated *Clostridium difficile* infection

	Univariate analysis			Multivariate analysis		
	OR	95% CI	p	OR	95% CI	p
Epidemiological characteristics						
Age ≥65 years	4.84	1.78 - 13.13	0.002	3.26*	1.08 - 9.78	0.035
Health-care-associated *vs.* community-associated and indeterminate infection	3.23	0.92 - 11.40	0.068	4.86*	0.59 - 40.04	0.141
Severe comorbidity (APACHE II CHP >0)	1.17	0.57 - 2.40	0.666	..	..	..
Liver cirrhosis (APACHE II)	0.53	0.06 - 4.56	0.562	..	..	..
Heart disease (APACHE II)	1.71	0.62 - 4.76	0.302	..	..	..
Pulmonary disease (APACHE II)	2.66	1.11 - 6.37	0.028	1.38*	0.48 - 4.02	0.543
Chronic dialysis (APACHE II)	0.29	0.04 - 2.35	0.248	..	..	..
Immunocompromised status (APACHE II)	0.92	0.39 - 2.17	0.850	..	..	..
Treatment for inflammatory bowel disease†	..	..	..	..	..	..
Use of an antibiotic not directed at *C. difficile* infection during previous month						
Aminopenicillin	2.69	0.69 - 10.51	0.156	2.39*	0.43 - 13.33	0.320
Aminopenicillin - β-lactamase inhibitor combination	1.81	0.80 - 4.06	0.153	1.18*	0.43 - 3.23	0.741
Antipseudomonal penicillin - β-lactamase inhibitor combination‡	..	..	..	..	..	..
Second-generation cephalosporin	0.53	0.14 - 1.97	0.343	..	..	..
Ceftazidime	1.34	0.52 - 3.46	0.546	..	..	..
Any cephalosporin	0.92	0.42 - 2.02	0.831	..	..	..
Carbapenem	1.29	0.42 - 4.00	0.657	..	..	..
Aminoglycoside	1.65	0.45 - 6.05	0.453	..	..	..
Old quinolone	1.41	0.57 - 3.53	0.459	..	..	..
New quinolone	3.45	1.07 - 11.06	0.038	2.57*	0.68 - 9.72	0.163
Any quinolone	2.29	1.03 - 5.09	0.043	..	..	..
Intravenous glycopeptide	1.95	0.61 - 6.20	0.257	..	..	..
Lincosamide	0.32	0.04 - 2. 79	0.303	..	..	..
Macrolide	2.69	0.80 - 9.00	0.108	4.60*	0.72 - 29.37	0.107
Co-trimoxazole	0.33	0.04 - 2.83	0.321	..	..	..
Episodes of infectionl in 8 weeks before current episode	0.77	0.27 - 2.19	0.621	..	..	..
***Clostridium difficile* infection characteristics**						
Duration of diarrhoea >1 week	0.55	0.23 - 1.32	0.182	..	..	..
Diarrhoea mixed with blood	1.06	0.33 - 3.42	0.928	..	..	..
Fever (temperature >38.5°C)	1.28	0.59 - 2.76	0.533	..	..	..
Ileus	2.84	0.73 - 11.08	0.132	..	..	..
Leukocyte count ≥15 × 10^9/L	1.50	0.67 - 3.35	0.324	..	..	..
Serum creatinine rise >50%	2.33	0.63 - 8.63	0.205	..	..	..
Bowel distension	2.06	0.38 - 11.25	0.405	..	..	..
Microbiological characteristics						
PCR-ribotype 027§	4.72	1.34 - 16.56	0.016	2.56¶	0.64 - 10.25	0.184
PCR-ribotype 078§	1.08	0.29 - 4.10	0.909	..	..	..
PCR-ribotype 014/020§	0.43	0.12 - 1.50	0.184	0.60¶	0.17 - 2.16	0.433
PCR-ribotype 015§	3.77	1.01 - 14.08	0.048	4.56¶	0.98 - 21.20	0.053
PCR-ribotype 018§	9.22	2.24 - 38.09	0.002	6.19¶	1.28 - 29.81	0.023
PCR-ribotype 023§	1.00	0.11 - 9.11	0.999	..	..	..
PCR-ribotype 056§	10.96	0.96 - 126	0.054	13.01¶	1.14 - 148.26	0.039
Presence of either or both binary toxin genes	1.09	0.46 - 2.54	0.847	..	..	..
Toxin A negative, toxin B positive strains *vs.* all other strains	0.69	0.08 - 6.08	0.739	..	..	..
Toxinotype III (including IIIb and IIIc) *vs.* all other toxinotypes	3.18	0.96 - 10.56	0.059	1.81¶	0.48 - 6.75	0.378

OR=odds ratio. APACHE II=acute physiology, age, chronic health evaluation version II. CHP=chronic health points. ..=data not available. *Adjusted for other variables: age ≥65 years, health-care association, pulmonary disease, previous use of aminopenicillin, previous use of aminopenicillin with β-lactamase inhibitor, previous use of a new quinolone, previous use of macrolide, PCR-ribotype 027, PCR-ribotype 014/020, and PCR ribotype 056. †No complicated *Clostridium diff cile* infection occurred in 16 patients treated for inflammatory bowel disease *versus* 44 cases of complicated *C. difficile* infection in 419 patients without inflammatory bowel disease. ‡No cases of complicated *C. difficile* infection occurred in 34 patients who received an antipseudomonal penicillin–β-lactamase inhibitor combination *versus* 43 cases of complicated *C. difficile* infection in 381 patients who did not receive drug combination. §*Versus* all other ribotypes. ¶Adjusted for other variables: age ≥65 years, health-care association, pulmonary disease, previous use of aminopenicillin, previous use of aminopenicillin with β-lactamase inhibitor, previous use of a new quinolone, previous use of macrolide.

Table 5 Determinants of recurrence of *Clostridium difficile* infection during follow-up

	Univariate			Multivariate		
	OR	95%CI	p	OR	95%CI	p
Epidemiological characteristics						
Age ≥65 years	1.91	1.08 - 3.37	0.026	1.86*	0.88 - 3.92	0.104
Health-care-associated *versus* community-associated and indeterminate	1.77	0.83 - 3.78	0.139	1.93*	0.59 - 6.35	0.278
Severe comorbidity (APACHE II CHP >0)	1.35	0.79 - 2.31	0.273	..	..	..
Liver cirrhosis (APACHE II)	0.50	0.11 - 2.33	0.375	..	..	..
Heart disease (APACHE II)	1.16	0.50 - 2.68	0.734	..	..	..
Pulmonary disease (APACHE II)	0.51	0.20 - 1.32	0.165	0.62*	0.20 - 1.95	0.417
Chronic dialysis (APACHE II)	2.04	0.79 - 5.26	0.139	2.23*	0.59 - 8.37	0.235
Immunocompromised status (APACHE II)	1.22	0.66 - 2.24	0.531	..	..	..
Treatment for inflammatory bowel disease†	..	..	..	..	..	..
Use of an antibiotic not directed at *C. difficile* infection during previous month						
Aminopenicillin	1.04	0.35 - 3.13	0.941	..	..	..
Aminopenicillin - β-lactamase inhibitor combination	1.17	0.60 - 2.28	0.643	..	..	..
Antipseudomonal penicillin - β-lactamase inhibitor combination	1.78	0.76 - 4.20	0.186	2.32*	0.79 - 6.82	0.125
Second-generation cephalosporin	0.62	0.26 - 1.43	0.261	..	..	..
Ceftazidime	2.25	1.17 - 4.29	0.015	2.48*	1.06 - 5.81	0.036
Any cephalosporin	1.11	0.63 - 1.94	0.721	..	..	..
Carbapenem	0.81	0.31 - 2.11	0.661	..	..	..
Aminoglycoside	1.60	0.59 - 4.28	0.354	..	..	..
Old quinolone	1.22	0.63 - 2.39	0.555	..	..	..
New quinolone	1.60	0.57 - 4.26	0.368	..	..	..
Any quinolone	1.35	0.73 - 2.47	0.335	..	..	..
Intravenous glycopeptide	1.73	0.71 - 4.20	0.228	..	..	..
Lincosamide	1.78	0.64 - 4.96	0.271	..	..	..
Macrolide	1.03	0.35 - 3.02	0.952	..	..	..
Co-trimoxazole	0.15	0.02 - 1.18	0.071	..	..	..
Episodes of *C. difficile* infection in 8 weeks before current episode	2.15	1.10 - 4.22	0.025	2.26*	1.03 - 4.96	0.041
***C. difficile* infection characteristics**						
Duration of diarrhoea >1 week	1.01	0.56 - 1.83	0.965	..	..	..
Diarrhoea mixed with blood	0.49	0.18 - 1.36	0.171	..	..	..
Fever (temperature >38.5°C)	1.17	0.71 - 2.06	0.572	..	..	..
Ileus	0.24	0.03 - 1.92	0.177	..	..	..
Leukocyte count ≥15 × 10^9/L	0.99	0.53 - 1.85	0.973	..	..	..
Serum creatinine rise >50%	0.90	0.30 - 2.69	0.850	..	..	..
Pseudomembranes‡	..	..	..	..	..	..
Ulceration	1.12	0.06 - 21.17	0.941	..	..	..
Colonic wall thickening	2.24	0.50 - 10.01	0.290	..	..	..
Pericolonic fat stranding	3.12	0.47 - 20.55	0.237	..	..	..
Bowel distension	0.60	0.16 - 2.24	0.445	..	..	..
Microbiological characteristics						
PCR-ribotype 027§	2.06	0.66 -6.43	0.211	..	..	..
PCR-ribotype 078§	1.62	0.67 -3.90	0.286	..	..	..
PCR-ribotype 014/020§	0.86	0.39 - 1.89	0.700	..	..	..
PCR-ibotype 015§	1.72	0.47 - 6.30	0.411	..	..	..
PCR-ibotype 018§	2.39	0.70 - 8.16	0.165	0.50¶	0.07 - 3.71	0.495
PCR-ibotype 023§	2.89	0.72 - 11.61	0.135	1.76¶	0.33 - 9.29	0.508
PCR-ibotype 056§	1.75	0.27 - 11.47	0.557	..	..	..
Presence of either or both binary toxin genes	1.63	0.89 - 2.97	0.113	..	..	..
Toxin A negative, toxin B positive strains *vs.* all other strains	0.69	0.14 - 3.46	0.654	..	..	..
Toxinotype III (including IIIb and IIIc) *vs.* all other toxinotypes	1.38	0.48 - 3.94	0.551	..	..	..

OR=odds ratio. APACHE II=acute physiology, age, chronic health evaluation version II. CHP=chronic health points. ..=data not available. *Adjusted for other: age ≥65 years, health-care association, pulmonary disease, chronic dialysis, previous use of antipseudomonal penicillin with β-lactamase inhibitor, previous use of ceftazidime, episodes of *C. difficile* infection 8 weeks before current episode, PCR-ribotype 018, PCR-ribotype 023, and presence of either or both binary toxin genes. †No recurrences in 19 patients with inflammatory bowel disease *versus* 83 recurrences in 419 patients without inflammatory bowel disease. ‡No recurrences in seven patients with pseudombranes *versus* two recurrences in 21 patients without pseudomembranes. §*Versus* all other ribotypes. ¶Adjusted for other variables: age ≥65 years, health-care association, pulmonary disease, chronic dialysis, previous use of antipseudomonal penicillin with β-lactamase inhibitor, previous use of ceftazidime and episodes of *C. difficile* infection 8 weeks before current episode.

Discussion

We have shown that the incidence of *C. difficile* infection and the distribution of causative PCR ribotypes differed greatly between hospitals in Europe; overall and attributable mortality were strikingly high. The strengths of this pan-European study are the large number of participating countries and hospitals, and a study design with a fixed 3-month follow-up. The high follow-up rate and the fact that patients with missing follow-up were younger, were more likely to be outpatients, and had less comorbidity than patients with follow-up, minimised the risk that cases of complicated infection were missed. If all patients with missing follow-up information had had an uncomplicated course, this factor would not have affected predictors for complicated infection.

This study has some limitations. First, selection of the hospitals in each country was left to the national coordinators, and the number of hospitals per country was small. Therefore, results derived from this sample of hospitals might not be representative of each country. Furthermore, some hospitals might have been selected because of outbreaks of *C. difficile* infection, thus introducing bias. Second, there might have been differences in physician awareness of infection between hospitals and countries. We note that the frequency of testing for infection varied up to 47 times between countries (as expressed by number of patients tested per 10,000 patient-days; table 1). Additionally, because there is no consensus on optimum testing for *C. difficile* infection, diagnostic (and culture) methods were not uniform. Third, detailed information for cases of infection was obtained only for the first ten patients enrolled in each hospital, which might have introduced bias if risk factors varied across hospitals. Furthermore, this low number might have led to under-representation of PCR ribotypes that caused outbreaks of infection in some hospitals.

Results from endoscopy or CT might be biased since these examinations tend to be triggered by a more severe course of disease. The proportion of patients with severe comorbidity might be overestimated because one of five items was sufficient to declare severe comorbidity, whereas if one item was scored missing, absence of severe comorbidity could not be declared.

Barbut and colleagues[21] reported a mean incidence of nosocomial *C. difficile* infection in 23 European hospitals of 2.45 per 10,000 patient-days (minimum to maximum range; 0.1-7.1), which is lower than the overall figure of 4.1 per 10,000 patient-days in our study. However, that study differed from ours in methodology. Reports from Denmark, Finland, Germany, Spain, and the UK[22–25] support the impression of an increase in incidence of *C. difficile* infection in Europe. PCR ribotypes identified by Barbut and colleagues[21] differed strikingly from those we identified. In their study, among isolates from 38 hospitals in 14 countries, PCR-ribotypes 001 and 014 were the most prevalent, followed by 027 and 020. Epidemic PCR-ribotype 027 was less prevalent in our study. By contrast, the prevalence of PCR-ribotypes 078 and 018 was increased. The high prevalence of PCR-ribotype 018 in our study is accounted for by its high prevalence in three Italian hospitals. Barbut and colleagues[21] reported that PCR-ribotype 078 was dominant only in Greece, whereas in our study it was the third most prevalent PCR ribotype. This increase of PCR ribotype-078 in Europe accords with findings for the Netherlands[26] and reports of PCR ribotype-078 in piglets with diarrhoea in the Netherlands and Spain.[27,28] Interestingly, human and animal isolates of PCR-ribotype 078 are genetically highly related, supporting the hypothesis that no interspecies barrier exists for *C. difficile* infection due to PCR-ribotype 078.[26] Research suggests that food products might play a part in interspecies transmission.[29,30] In one study, patients infected with PCR-ribotype 078 were younger than those infected with PCR-ribotype 027, but had a similar attributable mortality.[27] We could not show an association between PCR-ribotype 078 and complicated infection; however, patients with infection as a result of this ribotype (n=31) were more likely to have a rise in serum creatinine than were patients with other ribotypes (n=362, OR 3.20, 95% CI 1.08 - 9.49), and had a slightly higher mean leukocyte count.

Although we emphasise that *C. difficile* infection incidence rates of participating hospitals were not representative of national incidence rates, many hospitals with high rates of *C. difficile* infection were from countries in northern and central Europe. Most of these countries are thought to have low antibiotic consumption per head, even during the winter-respiratory-infection season.[31] Heightened awareness of *C. difficile* infection, as shown by the number of patients tested per 10,000 patient-days, might partly account for these differences in infection-incidence rates. Differences in the severity of illness of patients in hospital or those prescribed antibiotics might be other explanations. Patients admitted to high-incidence hospitals were more likely to have received aminopenicillins and first-generation and second-generation cephalosporins than were patients admitted to low-incidence hospitals.

Most risk factors for complicated or recurrent infection were consistent with those reported in previous studies. Old age,[32-34] previous hospital or nursing-home admission,[33] ileus,[33,34] and infection by PCR-ribotype 027[35] have been associated with complicated *C. difficile* infection. The use of certain antibiotics, especially fluoroquinolones, has been associated with infection by PCR-ribotype 027, and through this association with complicated or recurrent disease.[35,36] We did not find an association between the use of fluoroquinolones and complicated or recurrent disease, possibly because of the small number of infections resulting from PCR-ribotype 027 in our study. Alternatively, some confounding effects in earlier studies—notably data for antimicrobial prescribing in outbreak settings that might overestimate *C. difficile* infection risk associated with specific antibiotics—were not

as likely in our study. An association of PCR-ribotypes 018 and 056 with complicated infection has not been reported before. However, the number of complicated infections for which these associations were based was small. Old age[32,37] and a long cumulative duration of previous episodes of *C. difficile* infection[38] have been identified as predictors of recurrent infection. We could not confirm leucocytosis[33,34,37,39] as a strong predictor of complicated infection, possibly because we included leukocyte counts only from the week before the patients' inclusion, whereas in most studies the maximum leukocyte count during the course of the illness was examined. These findings underscore the importance of local surveillance to detect and control endemic and epidemic *C. difficile* infection.

Contributors

The study was designed by DWN, BHBB, MHW, and EJK, with support of DLM, on behalf of ECDC, and members of European Study group of *Clostridum difficile,* on behalf of European Society for Clinical Microbiology and Infectious Diseases. JSB and MR were responsible for PCR ribotyping and toxinotyping of strains, respectively. MPB did the study as principle coordinator, using support of DWN as principal investigator and EJK as microbiological coordinator. DLM helped in selecting national coordinators. BHBB and JTvD supervised clinical data collection and data analysis. MPB analysed the data and wrote the first draft of the article. All authors contributed substantially to the submitted version.

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Conflicts of interest

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