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Epidemiology of *Clostridium difficile* Infection and Risk Factors for Unfavorable Clinical Outcomes: Results of a Hospital-Based Study in Barcelona, Spain

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Prospective hospital-based surveillance for *Clostridium difficile*-associated disease (CDAD) was conducted in Barcelona (Spain) to describe the epidemiology of this condition and investigate the risk factors for an unfavorable outcome. All patients diagnosed with CDAD during 2009 were included. Using logistic regression modeling, we analyzed the potential risk factors associated with recurrent and complicated CDAD, defined as a need for colectomy or death within 30 days. There were 365 episodes of CDAD, yielding an incidence of 22.5 cases/10^5 person-years, 1.22 cases/10^3 hospital discharges, and 1.93 cases/10^4 patient-days. The main PCR ribotypes identified were 241 (26%), 126 (18%), 078 (7%), and 020 (5%). PCR ribotype 027 was not detected. Among the 348 cases analyzed, 232 (67%) patients were cured, 63 (18%) had a recurrence of CDAD, and 53 (15%) developed complicated CDAD. Predictors of complicated CDAD were continued use of antibiotics following CDAD diagnosis (odds ratio [OR], 2.009; 95% confidence interval [CI], 1.012 to 3.988; $P = 0.046$), Charlson comorbidity index score (OR, 1.265; 95% CI, 1.105 to 1.449; $P = 0.001$), and age (OR, 1.028; 95% CI, 1.005 to 1.053; $P = 0.019$). A leukocyte count of $>15 \times 10^3$ cells/ml (OR, 2.277; 95% CI, 1.189 to 4.362; $P = 0.013$), continuation of proton pump inhibitor (PPI) use after CDAD diagnosis (OR, 2.168; 95% CI, 1.081 to 4.347; $P = 0.029$), and age (OR, 1.021; 95% CI, 1.001 to 1.041; $P = 0.036$) were independently associated with higher odds of recurrence. The incidence of CDAD in Barcelona during 2009 was on the lower end of the previously described range for all of Europe. Our analysis suggests that the continuation of non-*C. difficile* antibiotics and use of PPIs in patients diagnosed with CDAD are associated with unfavorable clinical outcomes.

Clostridium difficile-associated disease (CDAD) is a potentially serious emerging condition. It is the most commonly recognized cause of health care-associated diarrhea, and it has also been associated with community acquisition among young and relatively healthy individuals without known predisposing factors (1, 2, 3, 4). *C. difficile* is found as a commensal or pathogen in the intestinal tracts of most mammals. Pet animals have been identified as reservoirs of *C. difficile* PCR ribotypes that can also infect humans. Moreover, PCR ribotype 078 is the most common *C. difficile* ribotype found in pigs and cattle and is now the third most common *C. difficile* ribotype found in human infections in Europe. Human and porcine strains of *C. difficile* are genetically identical in Europe, confirming that *C. difficile* infection is zoonotic and supporting the notion that animals are a reservoir for human infection (5, 6, 7).

The clinical spectrum of CDAD ranges from mild diarrhea to fulminant colitis in 3% to 8% of patients (8). Large outbreaks of CDAD have been reported in the United States and Canada, including the emergence of an epidemic hypervirulent strain (BI/NAP/027) (9, 10, 11). The incidence of this illness in nonoutbreak situations has been described less extensively (12, 13, 14, 15, 16).

The first data on the incidence of CDAD in Europe came from a survey performed in 2002 that estimated a mean incidence of 11 cases/10^4 hospital admissions (14). In Spain, a recent survey of laboratory diagnoses of CDAD estimated an annual incidence of 1.71 cases/10^3 hospital admissions (12). Local surveillance of *C. difficile* infection is important, not only to detect endemic and epidemic CDAD, but also to detect risk factors and enable the identification of patients at risk of acquiring severe CDAD. The data obtained can help clinicians optimize treatment and improve the outcome of this condition.

The aims of this study were to estimate the incidence and epidemiology of CDAD in Barcelona, to determine the ribotypes and toxin patterns of the isolated strains, and to identify the predictors of an unfavorable outcome, defined as complicated CDAD or a first recurrence of the disease.

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TABLE 1 Incidence of CDAD and characteristics of hospitals included in surveillance for *Clostridium difficile* infection, in Barcelona, Spain (2009)[a]

Hospital no.	No. of beds	No. of cases	No. of discharges	No. of patient-days	Incidences per 1,000 discharges (95% CI)	Incidence per 10,000 patient-days (95% CI)
1	1,290	125	47,859	380,479	2.61 (2.19–3.11)	3.29 (2.76–3.91)
2	900	46	23,918	323,630	1.92 (1.44–2.57)	1.42 (1.06–1.90)
3	700	53	48,213	273,112	1.10 (0.84–1.44)	1.94 (1.48–2.54)
4	620	72	33,928	205,938	2.12 (1.68–2.67)	3.50 (2.78–4.40)
5	450	3	12,360	46,260	0.24 (0.08–0.75)	0.65 (0.21–2.01)
6	362	3	20,616	90,710	0.15 (0.05–0.45)	0.33 (0.11–1.03)
7	306	11	1,842	75,125	5.97 (3.31–10.78)	1.46 (0.81–2.64)
8	280	13	15,771	87,853	0.82 (0.48–1.42)	1.48 (0.86–2.55)
9	270	4	19,100	68,285	0.21 (0.08–0.56)	0.59 (0.22–1.56)
10	263	5	15,279	69,415	0.33 (0.14–0.79)	0.72 (0.30–1.73)
11	192	5	6,722	48,645	0.74 (0.31–1.79)	1.03 (0.43–2.47)
12	175	15	21,203	70,000	0.71 (0.43–1.17)	2.14 (1.29–3.55)
13	142	2	10,601	42,275	0.19 (0.05–0.75)	0.47 (0.12–1.89)
14	139	0	10,463	42,375	0.00	0.00
15	120	1	6,345	33,692	0.16 (0.02–1.12)	0.30 (0.04–2.11)
Total	6,349	358	294,220	1,857,794	1.22 (1.10–1.35)	1.93 (1.74–2.14)

[a] CDAD, *Clostridium difficile*-associated disease.

MATERIALS AND METHODS

Study design and population. Active, prospective, hospital-based surveillance for CDAD was conducted in Barcelona, Spain (2009 local census indicated 1,621,537 inhabitants in the city) between 1 January and 31 December 2009. Fifteen major institutions participated, ranging in size from 120 to 1,290 beds and accounting for all hospitals in Barcelona where *C. difficile* testing is performed and where patients with acute illnesses are admitted. The participating clinical laboratories were periodically audited to ensure that all cases of *C. difficile* infection had been reported. Cases found after the audits were added to the analysis. A standardized questionnaire was prospectively completed by the attending physician of each patient and was carefully reviewed by the study coordinator (D.R.-P). Any inconsistency or contradiction found was double checked by the investigator at each hospital. Questionnaire contents included demographics, baseline comorbidity status measured by the Charlson comorbidity index, presence of different comorbidities (malignancy, diabetes mellitus, chronic renal failure, chronic cardiac or pulmonary disease, liver cirrhosis, or transplant recipient), known predisposing risk factors in the month preceding each patient's first positive *C. difficile* toxin result (antimicrobial treatment, use of proton pump inhibitors, laxatives, loperamide, parenteral or enteral feeding, and immunosuppressive treatments, including chemotherapy, corticosteroids, and/or immunomodulating drugs), clinical data concerning CDAD (diarrhea, abdominal pain, fever), biological markers measured at CDAD diagnosis (blood leukocyte count, creatinine, and albumin values), and outcomes.

There was no research-related contract with patients. Informed consent was not required because patients were treated according to the local standard of care and no additional clinical interventions were made based on the data collection process. All activity was in accordance with the Declaration of Helsinki and national and institutional standards.

Definitions. A CDAD case was defined as a surveillance-area resident with diarrhea (≥3 loose stools/day) or toxic megacolon who consulted at one of the 15 participating study hospitals and was diagnosed by a positive *C. difficile* toxin A or B test, the detection of a toxin-producing *C. difficile* organism in a stool sample by culture, or endoscopic or histopathologic evidence of pseudomembranous colitis (17).

Case patients were categorized by the setting in which *C. difficile* was likely acquired, according to the criteria given by McDonald et al. (17). Patients were followed up for ≥3 months after the diagnosis. Two episodes in the same patient were considered different events if they occurred >8 weeks apart.

The Charlson comorbidity index (18) was used to represent the cumulative burden of comorbid illnesses.

Outcomes. A cure was defined as the resolution of the symptoms associated with the initial episode of CDAD in the following 8 weeks. A designation of unfavorable outcome was established based on two situations: (i) development of complicated CDAD, defined by a need for colectomy or death within 30 days after onset, or (ii) recurrence, defined as an episode of CDAD (i.e., one that met the criteria of a CDAD case) occurring up to 8 weeks after the onset of a previous episode, provided that CDAD symptoms from the earlier episode had resolved with or without therapy (17). Admission to an intensive care unit was not included as a criterion for complicated CDAD, since the indications for admission varied considerably among the 15 participating hospitals due to their dissimilar characteristics.

Microbiological methods. Identification of samples for *C. difficile* toxin analysis was performed in each participating laboratory by stool specimen testing with an enzyme immunoassay (EIA) that detects both toxin A and toxin B. In all patients with unresolved diarrhea and negative EIA testing for *C. difficile*, the test was repeated in 48 to 72 h. In addition, the presence of toxin-producing *C. difficile* was sought in stool samples by toxigenic culture, which was performed in five of the centers listed in Table 1, including the four tertiary referral centers for adult patients located in Barcelona. Repeated samples from the same patient were excluded. Culture and antibiotic susceptibility studies were centralized and performed in four participating laboratories. MICs were determined by the Etest method (bioMérieux, Mercy l'Etoile, France). Susceptibility was defined according to the following breakpoints: amoxicillin-clavulanate (≤2 mg/liter), vancomycin (≤2 mg/liter), metronidazole (≤8 mg/liter), tigecycline (≤2 mg/liter), erythromycin (≤2 mg/liter), clindamycin (≤2 mg/liter), rifampin (≤1 mg/liter), ciprofloxacin (≤2 mg/liter), and moxifloxacin (≤2 mg/liter). The presence of toxins A and B and binary toxin genes was investigated by the detection of the *tcdA*, *tcdB*, *tcdC*, *cdtA*, and *cdtB* genes, using the methods described by Lemee et al. (19), Spigaglia et al. (20), and Braund et al. (21) Capillary gel electrophoresis-based PCR ribotyping was performed on available *C. difficile* isolates using a method modified from that of Indra et al. (22). Results were submitted to the WEBRIBO Web-based database (http://webribo.ages.at).

Statistical analysis. Incidence and age-specific rates were calculated using denominator data obtained from the 2009 local census. Hospital-specific incidence was calculated using the denominators obtained from the individual hospitals for the total number of patients discharged and

patient-days in 2009. Overall incidence was calculated using the denominators of summed discharges and patient-days to calculate pooled mean rates.

In reporting these data, categorical variables were expressed as percentages, and quantitative data as the median and interquartile range (IQR). The chi-square test or Fisher exact test was used to compare categorical variables and the Mann-Whitney U test was used for continuous variables. All statistical tests were two-tailed, and the threshold of statistical significance was a P value of <0.05. Predictors of complicated CDAD and recurrence were investigated using logistic regression analysis. To preserve the assumption of the independence of the observations, only the first episode of CDAD recorded for an individual patient was included in the analysis. Pediatric patients (age $<$ 15 years) were also excluded from the predictor analysis to avoid heterogeneity of the sample. For multivariate analysis of the predictors of complicated CDAD, candidate variables included those having a univariate significance at a P value of <0.10 and those believed to be clinically relevant based on our experience and data from the literature. Multivariate models were conducted in a sequential fashion. Predictors of recurrence were investigated using the same methodology. Variables were assessed for correlations and for significant interactions using the "chunk" test, a likelihood ratio test that compared the initial full model with a reduced model under the null hypothesis of no interaction terms. Odds ratios and 95% confidence intervals were used to quantify the strength of these associations. Final models were estimated using variables whose coefficients were stable across the range of possible models. Statistical analyses were performed with the SPSS software package, version 15.0 (SPSS, Chicago, IL).

RESULTS

During the study period, 362 patients presented with 365 episodes of CDAD, which yielded an average annual incidence of 22.5 cases/10^5 (Barcelona) population. Overall, the surveillance period included nearly 1.9 million patient-days and 294,220 hospital discharges, and the pooled mean CDAD rates for the 15 hospitals were 1.93 episodes/10^4 patient-days and 1.22 episodes/10^3 hospital discharges (Table 1). Incidences varied widely between the patient age groups, with the highest rate in patients aged $>$65 years (67 cases/10^5 population) and the lowest in children and adolescents aged $<$15 years (8.1 cases/10^5 population). The incidence in patients aged 15 to 65 years was 12.3 cases/10^5 population.

Complete data sets were available for analysis from 348 patients: the median age was 72 years (IQR, 57 to 82) and 174 were men (50%). Onset of *C. difficile* infection was in a health care facility in 247 (71%) cases, in the community in 49 (14%) cases, in the community but with health care-associated infection in 38 (11%) cases, and in an undetermined place in 14 (4%) cases. In health care facility-onset episodes, the median time between admission and CDAD diagnosis was 13 days (IQR, 7 to 26 days).

Demographics, patient comorbidities, risk factors, and clinical characteristics of CDAD are summarized in Table 2. Prior antibiotic use was documented in 290 (83%) patients and proton pump inhibitor (PPI) use in 260 (75%) (Table 2). The most commonly prescribed antibiotics administered within the month preceding the onset of diarrhea were penicillin associated with a beta-lactamase inhibitor in 134 (38%), fluoroquinolones in 100 (29%), cephalosporins in 86 (25%), carbapenems in 62 (18%), clindamycin in 18 (5%), and aminoglycosides in 15 (4%) patients. A single type of antibiotic was used in 140 (48%) patients, 2 or 3 different types were used in 128 (44%) patients, and $>$3 types were used in 22 (8%) patients. Following CDAD diagnosis, antibiotic treatment was discontinued in 189/290 (65%) patients, the same antimicrobial therapy was continued in 72 (25%) patients, and treatment was changed to another antibiotic in 29 (10%) patients.

TABLE 2 Demographics, comorbidities, clinical characteristics, and outcome of patients included in the study (N = 348 cases)

Characteristic[a]	Value
Median age (yr [IQR])	72 (57–82)
Male sex (*n* [%])	174/348 (50)
Median no. of days in hospital to CDAD onset (IQR)	13 (7–26)
Comorbidities	
Median Charlson comorbidity index score (IQR)	2 (1–4)
Malignancy (*n* [%])	102 (29)
Diabetes mellitus (*n* [%])	77 (22)
Chronic renal failure (creatinine > 1.4 mg/dl) (*n* [%])	57 (16)
Chronic cardiac disease (*n* [%])	53 (15)
Chronic obstructive pulmonary disease (*n* [%])	47 (13)
Peripheral vascular disease (*n* [%])	40 (12)
Dementia (*n* [%])	37 (11)
Liver cirrhosis (*n* [%])	32 (9)
Transplant recipient (*n* [%])	33 (9)
Inflammatory bowel disease (*n* [%])	6 (2)
No comorbidities (*n* [%])	61 (18)
Predisposing factors 1 mo preceding diagnosis	
Prior antibiotic treatment (*n* [%])	290 (83)
Prior PPI use (*n* [%])	260 (75)
Prior treatment with laxatives (*n* [%])	38 (11)
Enteral feeding (*n* [%])	33 (9.5)
Parenteral feeding (*n* [%])	26 (8)
Prior treatment with loperamide (*n* [%])	28 (8)
Prior immunosuppressive agent use (*n* [%])	109 (31)
Clinical and biological markers at CDAD diagnosis	
Diarrhea (*n* [%])	340 (98)
Ileus (*n* [%])	8 (2)
Abdominal pain (*n* [%])	139 (40)
Fever (*n* [%])	101 (29)
Median blood leukocyte count (cells/10^9 liters [IQR])	12,609 (7,000–15,800)
Median albumin value (g/dl [IQR])[b]	2.8/94 (2.3–3.3)
Median creatinine value (mg/dl [IQR])	0.97 (0.7–1.4)
Outcome (*n* [%])	
Patients cured	232 (67)
Recurrence of infection	63 (18)
Deaths within 30 days	49 (14)
Colectomies performed (*n* [%])	4 (1)

[a] IQR, interquartile range; CDAD, *Clostridium difficile*-associated disease; PPI, proton pump inhibitor.
[b] Albumin value was recorded for only 94 patients.

Antibiotic susceptibility was tested in all toxin-producing *C. difficile* strains isolated by culture, i.e., 154 total strains. All strains were susceptible to amoxicillin-clavulanate, metronidazole, vancomycin, and tigecycline. Resistance to rifampin, moxifloxacin, erythromycin, clindamycin, and ciprofloxacin was found in 24%, 43%, 49%, 74%, and 100% of isolates, respectively. Among 148 strains submitted to ribotyping, 116 (78%) were positive for both toxin A and toxin B, 29 (20%) were negative for toxin A and positive for toxin B, and 1 was positive for toxin A and toxin B1, and in 2 strains, toxin A or B expression could not be demonstrated. Binary toxin was present in 40 isolates (27%). No associations were found between binary toxin production and development of complicated CDAD (9/28 [32%] versus 31/120 [26%]; OR, 1.360; 95% CI, 0.557 to 3.319; $P = 0.498$) or recurrence (9/37 [24%] versus 31/111 [28%]; OR, 0.829; 95% CI, 0.352 to 1.956; $P = 0.669$). PCR ribotyping was performed on 147 strains, and 48 different PCR ribotypes were identified, with the most common

being ribotypes 241 (26%), 126 (18%), 078 (7%), 020 (5%), and 050 (3%). PCR ribotype 027 was not found in our analysis.

Antimicrobial drugs for *C. difficile* were given to 332/348 (95%) patients. Metronidazole was used in 297 (85%) cases, but was changed to oral vancomycin in 18 patients because of a lack of clinical response at a median of 7 (IQR, 5 to 10) days of treatment. Oral vancomycin was initially used in 33 cases due to severe disease (15 were treated concomitantly with intravenous [i.v.] metronidazole, 3 cases had rectal vancomycin added, and 2 cases had tigecycline and i.v. immunoglobulin added). Two patients were included in a blinded clinical trial on *C. difficile* treatment. In total, 51 (15%) patients were treated with oral vancomycin. These patients were more frequently transplant recipients (18% versus 8%, $P = 0.027$), patients with underlying malignancies (46% versus 26%, $P = 0.004$), patients receiving immunosuppressive treatment (50% versus 28%, $P = 0.002$), those with a higher Charlson comorbidity index score (median of 3 [IQR, 2 to 5] versus median of 2 [IQR, 1 to 3], $P = 0.003$), and those with more symptoms that would warrant admission to an intensive care unit (ICU) (12% versus 1%, $P = 0.001$). Complicated CDAD outcome was found significantly more frequently in patients who required vancomycin treatment at any time during the infection than in those who did not (30% versus 15%; $P = 0.008$).

Overall, 232 (67%) patients were cured, 63 (18%) had a recurrence, and 53 (15%) developed complicated CDAD (49 [14%] died and 4 [1%] required colectomy). On univariate analysis, numerous factors were found to be significantly associated with development of complicated CDAD (Table 3). Multivariate logistic regression modeling was used to assess whether the continued use of non-*C. difficile* antibiotics after the diagnosis of CDAD was associated with complicated CDAD. Blood albumin value was not included in the model because this variable was only recorded in 94 patients. After adjustment for age and Charlson scores, the continuation of non-*C. difficile* antibiotic treatment following CDAD diagnosis (OR, 2.009; 95% CI, 1.012 to 3.988; $P = 0.046$), Charlson score (OR, 1.265; 95% CI, 1.105 to 1.449; $P = 0.001$), and age (OR, 1.028; 95% CI, 1.005 to 1.053; $P = 0.019$) were independently associated with the development of complicated CDAD.

A separate analysis was performed to identify risk factors for a first CDAD recurrence in patients who did not die during *C. difficile* treatment or undergo colectomy (Table 4). A multivariate logistic regression model was fitted to assess whether the continued use of PPIs after CDAD diagnosis was associated with recurrence of infection. After adjusting for age, sex, and comorbid malignancy, a blood leukocyte count of $>15 \times 10^3$ cells/ml (OR, 2.277; 95% CI, 1.189 to 4.362; $P = 0.013$), continued use of PPIs after CDAD diagnosis (OR, 2.168; 95% CI, 1.081 to 4.347; $P = 0.029$), and age (OR, 1.021; 95% CI, 1.001 to 1.041; $P = 0.036$) were independently associated with a higher risk of recurrence.

DISCUSSION

The present study, which constitutes a broad survey of CDAD in the entire city of Barcelona in a nonoutbreak situation, found an incidence of CDAD for the year 2009 of 22.5 cases/10^5 population, 1.22 episodes/10^3 hospital discharges, and 1.93 episodes/10^4 patient-days. These incidence values are lower than the reported rates in the United States and Canada, (9, 11) and in countries that have experienced previous outbreaks, such as the Netherlands, Belgium, and the United Kingdom (9, 23, 24, 25). The estimated incidence in a European survey performed in 2005 (15) was 2.45 cases/10^4 patient-days (range, 0.13 to 7.1 cases/10^4 patient-days). Three years later, in November 2008, the European Centre for Disease Prevention and Control commissioned a prospective incidence survey of cases in 34 European countries (16) and reported a mean incidence of 4.1 cases/10^4 patient-days (range, 0 to 36.3 cases/10^4 patient-days).

Limited information is available on the incidence and epidemiology of CDAD in Spain. A national survey of CDAD laboratory diagnoses conducted in 2007 estimated an annual incidence of 1.71 cases/10^3 hospital admissions and 13.42 episodes/10^5 inhabitants (12). Our incidence rates are on the lower ends of the previously described ranges for other European countries, but they are consistent with the values reported by the VINCat Program (a surveillance program for nosocomial infections that was implemented in Catalonia in 2006), which estimated a median incidence of CDAD in Catalonia of 2.0 episodes/10^4 patient-days (26). These differences in CDAD incidence in Spain relative to those of other European countries might be explained, in part, by the absence of the PCR ribotype 027 clone in Spain (as has also been demonstrated in our series), which has been associated with multi-institutional outbreaks of CDAD (9, 10). In contrast, our annual incidence per 100,000 population is higher than the incidence reported by Alcalá et al. (12) (22.5 versus 13.42 cases/10^5 population). In an attempt to estimate the actual extent of CDAD in Spain, these authors had recently evaluated all unformed stool specimens (irrespective of the clinician's request) that were sent to a large group of microbiology laboratories on a single day (13). They concluded that CDAD was underdiagnosed in a high percentage of episodes and that the hospital-acquired rate of CDAD was 3.8 episodes/10^4 patient-days. In cases with only mild symptoms, CDAD might have been underdiagnosed because of a lack of clinical suspicion that would lead to testing, since most of these patients were cured once the causal agent (i.e., prior antibiotics) was removed. However, we agree with these authors in the fact that efforts must be made to improve the accuracy of CDAD diagnosis.

Considering the role of patient age, several studies have reported higher incidences in older patients (11, 27), but the true extent of CDAD in children (<18 years of age) remains under debate. Data from 22 hospitals across the United States have reported an incidence rate in pediatric patients of 7.24 to 12.80 cases/10^4 hospital discharges (2). Again, our results (8.1 cases/10^5 population in patients <15 years of age) are on the lower end of this range.

Although CDAD has classically been associated with hospital acquisition (27), it is increasingly recognized that some cases are community acquired (2, 3, 4) and that the risk factors for community-acquired CDAD overlap those of health care facility-associated infection (4). In our series, 14% of cases were community acquired, representing an incidence of 3.1/10^5 person-years, which is clearly lower than the incidence of community-acquired CDAD described in the United States (4). These differences might be partly explained by the fact that community-acquired cases with mild symptoms are likely underdiagnosed and are self-limited.

With regard to the ribotypes found in our study, a previously unreported high prevalence of ribotype 241 was observed. This discovery might be a clue indicating an upcoming outbreak, but a clonal spread of one strain would need to be proved. Given the

TABLE 3 Univariate and multivariate predictors of development of complicated CDAD ($N = 335$ patients)

Variable[a,b]	Patients with:		Univariate analysis		Multivariate analysis	
	Uncomplicated CDAD	Complicated CDAD	OR (95% CI)	*P*	OR (95% CI)	*P*
Age (yr)*[c]	72 (57–81)	77 (64–85)	1.03 (1.007–1.05)	0.010	1.028 (1.005–1.053)	0.019
Male sex	141/288 (49)	26/47 (55)	1.29 (0.69–2.40)	0.42		
Community-acquired CDAD	46/288 (16)	1/47 (2)	0.11 (0.01–0.85)	0.03		
Comorbidities						
Charlson comorbidity Index score*	2 (1–3)	3 (2–6)	1.28 (1.13–1.47)	<0.001	1.265 (1.105–1.449)	0.001
Malignancy	83/288 (29)	18/47 (38)	1.53 (0.81–2.91)	0.19		
Diabetes mellitus	62/288 (21)	15/47 (32)	1.71 (0.87–3.35)	0.12		
Chronic renal failure	42/288 (18)	15/47 (32)	2.75 (1.37–5.50)	0.004		
Chronic cardiac disease	43/288 (15)	9/47 (19)	1.35 (0.61–2.99)	0.46		
Chronic obstructive pulmonary disease	41/288 (14)	6/47 (13)	0.88 (0.35–2.21)	0.79		
Peripheral vascular disease	34/288 (12)	6/47 (13)	1.09 (0.43–2.76)	0.85		
Dementia	31/288 (11)	6/47 (13)	1.21 (0.48–3.09)	0.68		
Liver cirrhosis	21/288 (7)	6/47 (13)	1.86 (0.71–4.88)	0.21		
Transplant recipient	23/288 (8)	4/47 (8)	1.07 (0.35–3.25)	0.90		
Predisposing factors 1 mo preceding diagnosis						
Prior antibiotic treatment	240/288 (83)	40/47 (85)	1.14 (0.48–2.71)	0.76		
Total length of AB treatment prior to CDAD diagnosis*	12 (7–21)	11 (7–20)	1.004 (0.97–1.04)	0.82		
Continued antibiotic use after CDAD diagnosis	62/288 (21)	17/47 (36)	2.066 (1.07–3.99)	0.02	2.009 (1.012–3.988)	0.046
Prior PPI use	217/286 (76)	38/47 (81)	1.34 (0.62–2.91)	0.45		
PPI use after CDAD diagnosis	190/286 (66)	34/47 (72)	1.32 (0.66–2.62)	0.42		
Prior treatment with loperamide	27/288 (9)	1/47 (2)	0.21 (0.03–1.58)	0.09		
Prior treatment with laxatives	33/288 (11)	4/47 (8)	0.72 (0.24–2.13)	0.55		
Enteral feeding	21/288 (7)	7/47 (15)	2.225 (0.89–5.57)	0.81		
Parenteral feeding	18/288 (6)	4/47 (8)	1.39 (0.45–4.32)	0.56		
Prior immunosuppressive agent use	86/288 (30)	15/47 (32)	1.10 (0.57–2.14)	0.78		
Clinical and biological markers at CDAD diagnosis						
Abdominal pain	117/288 (41)	17/47 (36)	0.83 (0.44–1.57)	0.56		
Fever	85/288 (29)	12/47 (25)	0.82 (0.40–1.65)	0.57		
Blood leukocyte count (cells/ml)*	10,600 (7,000–15,725)	12,510 (7,820–18,025)	1.00 (0.999–1.000)	0.18		
Albumin value (g/dl)*	2.82 (2.47–3.26)	2.11 (1.60–2.75)	0.22 (0.07–0.67)	0.008		
Creatinine value (mg/dl)*	0.93 (0.70–1.30)	1.14 (0.93–1.85)	1.18 (0.90–1.56)	0.23		
CDAD treatment						
No specific CDAD treatment[d]	276/288 (96)	43/47 (91)	0.47 (0.14–1.52)	0.19		
First CDAD treatment with metronidazole	249/288 (87)	36/47 (77)	0.49 (0.23–1.06)	0.07		
First CDAD treatment with vancomycin[e]	26/288 (9)	7/47 (15)	1.84 (0.75–4.54)	0.18		

[a] All quantitative variables (those indicated with an asterisk) are expressed as the median and interquartile range (IQR), while the remaining variables are reported as the absolute number and percentage.
[b] CDAD, *Clostridium difficile*-associated disease; AB, antibiotic; PPI, proton pump inhibitor.
[c] Pediatric patients ($n = 13$) were excluded from the analysis.
[d] Two patients were included in a blinded clinical trial on *C. difficile* treatment, and we did not know which CDAD treatment they received.
[e] If first CDAD treatment included both metronidazole and vancomycin, the patient was included in the vancomycin group.

previously described situation of a low incidence of CDAD in Catalonia, these data might be useful for monitoring changes in type prevalence rates and therefore warrants further study. We also observed a high prevalence of ribotype 126, which is highly related to ribotype 078 and has been reported in piglets and calves and their immediate environments (7, 28, 29, 30, 31, 32). At this time, there are no available data on ribotype 126 distribution in animals in Spain and, again, the zoonotic potential of this ribotype should be investigated.

In our series, all the isolates studied were fully susceptible to metronidazole and vancomycin; hence, these antibiotics seem appropriate for empirical CDAD treatment. A poorer outcome and higher recurrence rates have been reported with metronidazole use in severe episodes (33, 34), and for this reason, in standard hospital practice, the sickest patients are treated with vancomycin, as is recommended in recent guidelines (35, 35a). In our series, patients who needed vancomycin treatment were predictably sicker, had greater severity of comorbid conditions, and ulti-

TABLE 4 Univariate and multivariate predictors of recurrent CDAD ($N = 317$ patients)

Variable[a,b]	Patients with:		Univariate analysis		Multivariate analysis	
	No recurrences ($N = 255$)[f]	Recurrences ($N = 62$)[g]	OR (95% CI)	*P*	OR (95% CI)	*P*
Age (yr)*	71 (57–81)	75 (66–82)	1.024 (1.005–1.042)	0.012	1.021 (1.001–1.041)	0.036
Male sex	125/255 (49)	36/62 (58)	1.44 (0.82–2.52)	0.20		
Community-acquired CDAD	38/255 (15)	9/62 (15)	0.97 (0.44–2.13)	0.94		
Comorbidities						
Charlson Comorbidity Index score*	2 (1–4)	2 (1–3)	0.94 (1.82–1.08)	0.39		
Malignancy	81/255 (32)	13/62 (21)	0.57 (0.29–1.11)	0.095		
Diabetes mellitus	54/255 (21)	17/62 (27)	141 (0.75–2.65)	0.29		
Chronic renal failure	41/214 (16)	11/62 (18)	1.13 (0.54–2.34)	0.75		
Chronic cardiac disease	34/255 (13)	14/62 (23)	1.89 (0.94–3.80)	0.07		
Chronic obstructive pulmonary disease	33/255 (13)	11/62 (18)	1.45 (0.69–3.06)	0.33		
Peripheral vascular disease	31/255 (12)	7/62 (11)	0.92 (0.38–2.12)	0.85		
Dementia	27/255 (11)	7/62 (11)	1.07 (0.44–2.59)	0.87		
Liver cirrhosis	20/255 (8)	3/62 (5)	0.59 (0.17–2.08)	0.41		
Transplant recipient	23/255 (9)	4/62 (9)	0.69 (0.23–2.09)	0.52		
Predisposing factors 1 mo preceding diagnosis						
Prior antibiotic treatment	210/255 (82)	53/62 (86)	1.26 (0.58–2.74)	0.56		
Total length of AB treatment prior to CDAD diagnosis*	12 (7–21)	13 (9–21)	0.99 (0.96–1.03)	0.95		
Continued antibiotic treatment after CDAD diagnosis	60/255 (24)	11/62 (18)	0.70 (0.34–1.43)	0.33		
Prior PPI use	190/253 (75)	50/62 (81)	1.38 (0.69–2.76)	0.36		
PPI use after CDAD diagnosis	160/253 (63)	48/62 (77)	1.99 (1.043–3.81)	0.035	2.168 (1.081–4.347)	0.029
Prior treatment with loperamide	21/255 (8)	7/62 (11)	1.42 (0.57–3.50)	0.45		
Prior treatment with laxatives	30/255 (12)	6/62 (10)	0.80 (0.32–2.02)	0.64		
Enteral feeding	19/255 (8)	7/62 (11)	1.58 (0.63–3.95)	0.32		
Parenteral feeding	17/255 (7)	3/62 (5)	0.71 (0.20–2.51)	0.59		
Prior immunosuppressive agent use	78/255 (31)	16/62 (30)	0.79 (0.42–1.48)	0.46		
Clinical and biological markers at CDAD diagnosis						
Abdominal pain	101/255 (40)	24/62 (39)	0.96 (0.54–1.70)	0.90		
Fever	74/255 (29)	17/62 (27)	0.92 (0.49–1.72)	0.80		
Blood leukocyte count (cells/ml)*	10,235 (7,012–14,577)	12,675 (7,362–21,200)	1.00 (1.000–1.000)[c]	0.001		
Blood leukocyte count $> 15 \times 10^3$ cells/ml*	50/244 (20)	23/58 (40)	2.55 (1.39–4.70)	0.002	2.277 (1.189–4.362)	0.013
Median albumin value (g/dl)*	2.8 (2.3–3.2)	2.7 (2.5–3.1)	1.03 (0.45–2.35)	0.94		
Creatinine value (mg/dl)*	0.93 (0.7–1.4)	1.03 (0.76–1.64)	1.14 (0.88–1.48)	0.31		
CDAD treatment						
No specific CDAD treatment[d]	241/255 (95)	62/62 (100)	1.25 (1.19–1.33)	0.06		
First CDAD treatment with metronidazole	216/255 (85)	56/62 (90)	1.68 (0.68–4.18)	0.26		
First CDAD treatment with vancomycin[e]	24/255 (9)	5/62 (8)	0.84 (0.31–2.31)	0.74		

[a] All quantitative variables (those indicated with an asterisk) are expressed as the median and interquartile range (IQR), while the remaining variables are reported as the absolute number and percentage.
[b] CDAD, *Clostridium difficile*-associated disease; AB, antibiotic; PPI, proton pump inhibitor.
[c] Blood leukocyte count: 95% CI, 1.000020218563 to 1.000080793415.
[d] Two patients were included in a blinded clinical trial on CD treatment, and we did not know which CDAD treatment they received.
[e] If first CDAD treatment included both metronidazole and vancomycin, the patient was included in the vancomycin group.
[f] Patients with no recurrences comprised 80% of the total number of patients.
[g] Patients with recurrences comprised 20% of the total number of patients.

mately, had a higher percentage of complicated outcome than those receiving metronidazole. All the strains were resistant to ciprofloxacin, and 43% were resistant to moxifloxacin. These results are similar to the findings of Zaiss et al. (36), who reported frequent fluoroquinolone resistance in various *C. difficile* ribotypes, but not metronidazole or vancomycin resistance.

Of the total, 53 of our patients had a complicated CDAD outcome, and after pediatric patients were excluded, 47 patients were

included in the analysis of predictive factors. Continued use of non-*C. difficile* antimicrobial treatment following CDAD diagnosis, Charlson comorbidity index score, and age were independently associated with development of complicated CDAD. Age >65 years has been related to all unfavorable outcomes and an increased risk of recurrent CDAD (37, 38, 39). Older patients have a greater number of comorbid conditions, and they often live in long-term facilities or have experienced previous prolonged hospitalizations, which facilitates *C. difficile* acquisition (27). In addition, it has been speculated that the influence of older age probably reflects their weaker immune response against *C. difficile* and its toxins, as well as changes in gut microbiota composition due to changes in gut physiology and function associated with aging (40).

In our study, the continued use of non-*C. difficile* antibiotics after CDAD diagnosis was significantly associated with the development of complicated CDAD. Prior treatment with antimicrobials is considered the main risk factor for CDAD, and continued antibiotic treatment has been associated with a poor clinical course of this condition (2, 9, 25, 41, 42). Our results confirm previous observations and favor judicious antibiotic use to improve the outcome of CDAD. Although it would have been a logical finding, we did not see a significant association between recurrence of infection and the continued use of non-*C. difficile* antibiotics after CDAD diagnosis, likely because of the small sample size: only 14 of the 63 patients presenting with a recurrence continued with non-*C. difficile* antimicrobial therapy.

One finding worth highlighting is that continued use of PPIs after the diagnosis of CDAD was associated with recurrence of infection. In previous studies, PPI therapy was suggested to be a risk factor for acquiring CDAD, because the colonization barrier against vegetative forms of *C. difficile* would be decreased (2, 43, 44, 45, 46). Several studies have examined the prognostic significance of gastric acid suppression in CDAD. Some have reported an association of PPI use with increased severity of CDAD and mortality (39), and others with an increased risk of recurrence (47, 48). In contrast, Henrich et al. (38) found no association between gastric acid suppression and severe CDAD. Our results are consistent with those showing a link between PPI use and an increased risk of recurrence. Although *C. difficile* spores are acid resistant, vegetative forms are susceptible to acidity (47). The biological plausibility for an association between PPI use and an increased risk of persistent and recurrent *C. difficile* infection might be associated with the proliferation of bacteria, and therefore, of the vegetative forms of *C. difficile* in a previously sterile stomach. Undigested vegetative cells are allowed to pass into the distal gastrointestinal tract, and this may predispose a person to CDAD persistence (recurrence).

It is likely that confounding variables interact in these analyses. As Huttunen and Aittoniemi recently pointed out (49), PPIs are often given to severely ill patients who have greater severity of comorbid conditions and often require antibiotics, and these factors in themselves might be risk factors for CDAD. Based on our results and previous observations, we believe that PPIs should be discontinued whenever possible in patients with CDAD.

Blood leukocyte count, which has been significantly associated with severe CDAD in previous studies (11, 38, 50), likely reflects the severity of colon inflammation. Our study supports the value of this parameter as a predictor of a first recurrence, and therefore, we advocate for routine monitoring of leukocyte count in CDAD patients.

The observations in this study are subject to limitations. First, due to the fact that case reporting was hospital based, we may have missed some less-symptomatic community-acquired cases. Second, the Acute Physiology and Chronic Health Evaluation II (APACHE II) severity of illness score could not be recorded, and we were unable to adjust the multivariate analysis for this variable. Lastly, CDAD might have been underdiagnosed due to low clinical suspicion or insufficiently sensitive diagnostic methods. Sample analysis for *C. difficile* toxins was performed in each participating laboratory by EIA, which currently is not considered an optimal diagnostic method. However, we believe that the clinical impact of this factor is low: first, because in all patients with unresolved diarrhea and negative EIA testing for *C. difficile*, the test was repeated in 48 to 72 h, and second, because toxin-producing *C. difficile* was additionally investigated in stool samples by toxigenic culture in the referral centers included in the study. All unresolved and complicated cases were ultimately admitted to these centers, where the diagnosis would likely have been established. In contrast, mild cases detected by more-sensitive techniques alone are often self-limiting once the causal agent is removed, and patients are less likely to develop a complication, as has been recently demonstrated by Longtin et al. (51). The strengths of this study are the prospective consecutive data collection, which limits the likelihood of selection bias, and patient management by a specialized team at all participating centers.

In conclusion, the 2009 incidence rates of CDAD in Barcelona were on the lower end of the range of previously described rates in Europe. Continued use of non-*C. difficile* antibiotics after CDAD diagnosis, age, and Charlson comorbidity index score were predictors of the development of complicated CDAD, whereas continued PPI use, leukocyte count $>15 \times 10^3$ cells/ml, and age were predictors of recurrence. Our findings identify useful clinical and laboratory markers for patients at risk of an unfavorable CDAD outcome, and they support the careful scrutiny of antimicrobial use and PPI continuation once CDAD is diagnosed to improve the outcome of this illness.

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