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Original article

Risk factors for recurrence in patients with *Clostridium difficile* infection due to 027 and non-027 ribotypes

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ABSTRACT

Objectives: Our objective was to evaluate factors associated with recurrence in patients with 027+ and 027− *Clostridium difficile* infection (CDI).

Methods: Patients with CDI observed between January and December 2014 in six hospitals were consecutively included in the study. The 027 ribotype was deduced by the presence of *tcdB*, *tcdB*, *cdt* genes and the deletion Δ117 in *tcdC* (Xpert® *C. difficile*/Epi). Recurrence was defined as a positive laboratory test result for *C. difficile* more than 14 days but within 8 weeks after the initial diagnosis date with reappearance of symptoms. To identify factors associated with recurrence in 027+ and 027− CDI, a multivariate analysis was performed in each patient group. Subdistributional hazard ratios (sHRs) and 95% confidence intervals (95%CIs) were calculated.

Results: Overall, 238 patients with 027+ CDI and 267 with 027− CDI were analysed. On multivariate analysis metronidazole monotherapy (sHR 2.380, 95%CI 1.549–3.60, p <0.001) and immunosuppressive treatment (sHR 3.116, 95%CI 1.906–5.090, p <0.001) were factors associated with recurrence in patients with 027+ CDI. In this patient group, metronidazole monotherapy was independently associated with recurrence in both mild/moderate (sHR 1.894, 95%CI 1.051–3.410, p 0.033) and severe CDI (sHR 2.476, 95%CI 1.281–4.790, p 0.007). Conversely, non-severe disease (sHR 3.704, 95%CI 1.437–9.524, p 0.007) and absence of chronic renal failure (sHR 16.129, 95%CI 2.155–125.000, p 0.007) were associated with recurrence in 027− CDI.

Conclusions: Compared to vancomycin, metronidazole monotherapy appears less effective in curing CDI without relapse in the 027+ patient group, independently of disease severity. **M. Falcone, Clin Microbiol Infect 2018;▪:1**

Introduction

Clostridium difficile infection (CDI) is the most common cause of healthcare-associated diarrhoea [1]. It is associated with an increased length of hospital stay, readmission and mortality rates,

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and an excess of healthcare-related costs, especially when recurrence occurs [2]. Unfortunately, a recurrence occurs in approximately 19–20% of patients following a first episode of CDI, and it significantly increases the mortality rate [3,4]. Thus, the recent development of new therapeutic agents aims to reduce the recurrence rate in patients with CDI [5,6].

The global incidence of CDI has markedly increased in the last decades, and a rise in severe episodes has been reported worldwide [7–9]. This phenomenon has been partially attributed to the emergence of a hypervirulent strain, variously called North American pulsed-field gel electrophoresis type 1 (NAP1) strain, PCR ribotype 027, or restriction endonuclease analysis (REA) group BI, according to the detection method used [10,11]. Despite conflicting data, an association between 027 strain and poor outcome has recently been reported [12,13]. Moreover, infection by BI/NAP1/027 strain increases the risk of recurrence [14,15]. However, few studies have examined risk factors for recurrence in patients with initial infection due to a 027 strain in a non-epidemic setting.

Materials and methods

The aim of our study is to identify factors associated with recurrence in patients with CDI due to presumptive 027 (027+) and non-027 (027−) strains.

Setting

This observational study was conducted between January and December 2014 in the University Hospital Policlinico Umberto I – 'Sapienza' in Rome, which was designed as the coordinating centre of the study; CDI episodes were collected from five other large tertiary hospitals: San Giovanni Addolorata hospital Rome; Azienda Ospedaliera Sant'Andrea – 'Sapienza' University, Rome; Policlinico Tor vergata – 'Tor Vergata' University, Rome; Policlinico Gemelli – Catholic University, Rome; and Santa Maria Goretti hospital – 'Sapienza' University Polo Pontino, Latina. The involved hospitals care for a local population of 2.4 million people with a total number of 4631 beds. The local ethics committee approved the study.

Patients

We included patients with a first episode of CDI that met the definition criteria. CDI episodes occurring in all wards of hospitalization were included. We excluded from the study patients with unavailable data from the first CDI episode [16]. CDI was defined as (a) the presence of diarrhoea (passage of three or more unformed stools in ≤24 consecutive hours) and (b) a stool test result positive for the presence of toxigenic *C. difficile* or its toxins, or colonoscopic or histopathological findings demonstrating pseudomembranous colitis [17–20].

Data collection and study definitions

Based on records from microbiology laboratories of the involved centres, all cases with diarrhoea with positive assay for *C. difficile* were initially identified. The microbiological identification of *C. difficile* was performed in each hospital (six laboratories) by stool specimen testing with the commercial methods, using enzyme immunoassays (EIAs) combining detection of *C. difficile* glutamate dehydrogenase (GDH) and toxin A/B antigens. Presumptive ribotype 027 was deducted by the Cepheid Xpert *C. difficile*/Epi assay, which is a multiplex real-time polymerase chain reaction (PCR) that detects *tcdB*, the binary toxin gene (*cdt*), and the *tcdC* gene deletion at nt 117 (Δ117). Cases whose strain typing results were unavailable were excluded from the study.

All identified CDI cases subsequently underwent a full medical record review to collect information on demographics, place of acquisition, receipt of any immunosuppressive and antibiotic therapy during 14 days prior to infection, and clinical comorbidities. The Charlson comorbidity index was also calculated [21].

First episodes of CDI were classified as community-associated if a positive specimen was collected in an outpatient setting or ≤3 calendar days after hospital admission with no documented overnight stay in a healthcare facility (i.e., hospital or nursing home) in the previous 12 weeks. All the other cases were defined as healthcare-associated infections and were further classified into three subgroups: community-onset healthcare-associated if a positive specimen was collected in an outpatient setting or ≤3 calendar days after hospital admission from a private residence and documented overnight stay in a healthcare-facility (i.e., hospital or nursing home) in the previous 12 weeks; hospital-onset if a *C. difficile*-positive specimen was collected >3 calendar days after hospital admission or in a long-term acute-care hospital; nursing-home onset if a positive specimen was collected in a nursing home or from a nursing-home resident either in an outpatient setting or within 3 days after hospital admission [1].

All CDI cases were followed up for at least 8 weeks after a first episode of CDI with a site visit or, when that was not possible, through phone contact. Cure was defined as resolution of symptoms without recurrence in the 8 weeks following the diagnosis. Recurrent CDI was defined as another positive laboratory test result for *C. difficile* more than 14 days but ≤56 days after the initial diagnosis date in a patient with reappearance of symptoms [22].

Severity of CDI was defined according to criteria in the joint practice guidelines of the Society for Healthcare Epidemiology of America and the Infectious Diseases Society of America [18]. Patients with unexplained leucocytosis (white blood cell count ≥15,000/μL not attributable to other clinical conditions or comorbidities) or elevated serum creatinine level ≥1.5 times the baseline value (defined as the mean serum creatinine value in the 90 days before CDI) within 4 days of the CDI diagnosis date were considered to have severe CDI [20].

Therapy for the first CDI was coded in three categories: vancomycin monotherapy, metronidazole monotherapy, and vancomycin–metronidazole combination therapy. In order to assess the effects of the vancomycin dosage, we also evaluated a five-category variable in which vancomycin was separated in standard and high dosages (vancomycin monotherapy standard dosages, vancomycin monotherapy high dosages, metronidazole monotherapy, metronidazole plus vancomycin standard dosages, metronidazole plus vancomycin high dosages).

Statistical analysis

Incidence of CDI was expressed as cases per 10,000 patient-days. Incidence of recurrence was evaluated by means of Gray cumulative incidence estimator, taking into account the competing risk of death.

To evaluate differences between patients with CDI due to the 027 strain and those with episodes caused by non-027 strains, the study population was divided into two study groups: 027+ CDI and 027− CDI.

Data are expressed as the mean ± standard deviation or median (interquartile range, IQR) for continuous variables, and the number of cases (percentage) for categorical variables. All variables were tested for normality using the non-parametric Kolmogorov–Smirnov test. Continuous variables were compared by Student t-test for independent samples, for normally distributed variables, or by the Kruskal–Wallis test in cases of variables with a skewed distribution.

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Categorical variables were evaluated using the χ-square test or Fisher exact test when appropriate.

Since in our study an event (death) that precludes the occurrence of the event of interest (recurrence) was present, a competing risk analysis was performed using the subdistribution hazard model. Predictors of the first CDI recurrence were determined by Fine and Gray regression models for subdistributional hazards in each study group [23]. The final multivariate models were selected as the model with the lowest Akaike information criterion values. Variables associated with recurrence ($p < 0.05$) and non-correlated at the univariate analysis and those with clinical relevance were considered for the multivariate model.

Subdistributional hazard ratios (sHR) and 95%CIs were calculated to evaluate the strength of any association.

All data were statistically analysed using two commercially available statistical software packages (SPSS, version 20.0; SPSS Inc., Chicago, IL and R, version 3.4.2; R development core team, Wien, Austria). All tests were two-tailed, and a p value of 0.05 was considered statistically significant.

Results

From January to December 2014, 717 episodes of CDI were observed. CDI incidence was 4.2 cases/10,000 patient-days during the study period. Table S-1 shows CDI incidence for each hospital. No clusters were observed during the study period.

A diagram describing the study flow is shown in Fig. 1. Strain typing results were available for 563 of 717 total CDI cases identified in the study period; of these, 238 patients with a documented first episode of 027 CDI constituted the final group of 027+ CDI. The proportion of 027+ CDI was 49.4% in the Policlinico Umberto I hospital, 51.2% in the Torvergata University, 42.5% in the Sant'Andrea hospital, 41.7% in the Catholic University and 64.7% in the Santa Maria Goretti hospital. Conversely, 267 patients constituted the final group of 027− CDI.

Table 1 shows the comparison between 027+ and 027− groups. Overall, 48 (20.2%) patients with 027+ CDI and 15 (5.6%) patients with 027− CDI died during the 30 days after CDI diagnosis. The crude recurrence rate in the entire population was 20% (101 cases). The length of hospital stay was higher in patients in whom a recurrence developed (36 (IQR 32–59) days) than in those who did not (31.5 (IQR 20–37) days, $p < 0.001$). Compared to patients with CDI caused by non-027 strains, those with presumptive 027 infection had significantly higher crude recurrence rates (30.3% versus 10.9%, $p < 0.001$). Taking into account the competing risk of death, the recurrence rate among patients with 027+ and 027− CDI were 48.3% and 38.3%, respectively ($p < 0.001$).

Table 2 shows the comparison of patients with recurrence and those who were cured after the first CDI, according to the presumptive ribotype. The majority of recurrence episodes occurred during hospitalization in both the 027+ group (84.5%) and 027− group (82.8%). The group of patients treated with vancomycin plus metronidazole therapy had the lowest rate of recurrence (7.7% in 027+ CDI and no recurrence in 027− CDI). Baseline characteristics of these patients are shown in the Supplementary material Table S-2.

As described in Table 3, on multivariate analysis the use of metronidazole monotherapy compared to vancomycin (sHR 2.380, 95%CI 1.549–3.60, $p < 0.001$) and the receipt of immunosuppressive treatment (sHR 3.116, 95%CI 1.906–5.090, $p < 0.001$) were factors independently associated with recurrence in patients with 027+ CDI. This result was confirmed also when we considered five possible different treatments (low-dose vancomycin, high-dose vancomycin, metronidazole monotherapy, low-dose vancomycin plus metronidazole, high-dose vancomycin plus metronidazole), with an sHR of 2.69 (95%CI 1.00–7.27, p 0.01) for metronidazole monotherapy. Conversely, as shown in Table 4, on multivariate analysis lack of chronic renal failure (sHR 16.129, 95%CI 2.155–125.000, p 0.007) and non-severe CDI (sHR 3.704, 95%CI 1.437–9.524, p 0.007) were factors associated with recurrence in patients with CDI due to non-027 strains. Conversely, metronidazole monotherapy was not associated with recurrence in this patient group (sHR 1.896, 95%CI 0.974–3.690, p 0.060).

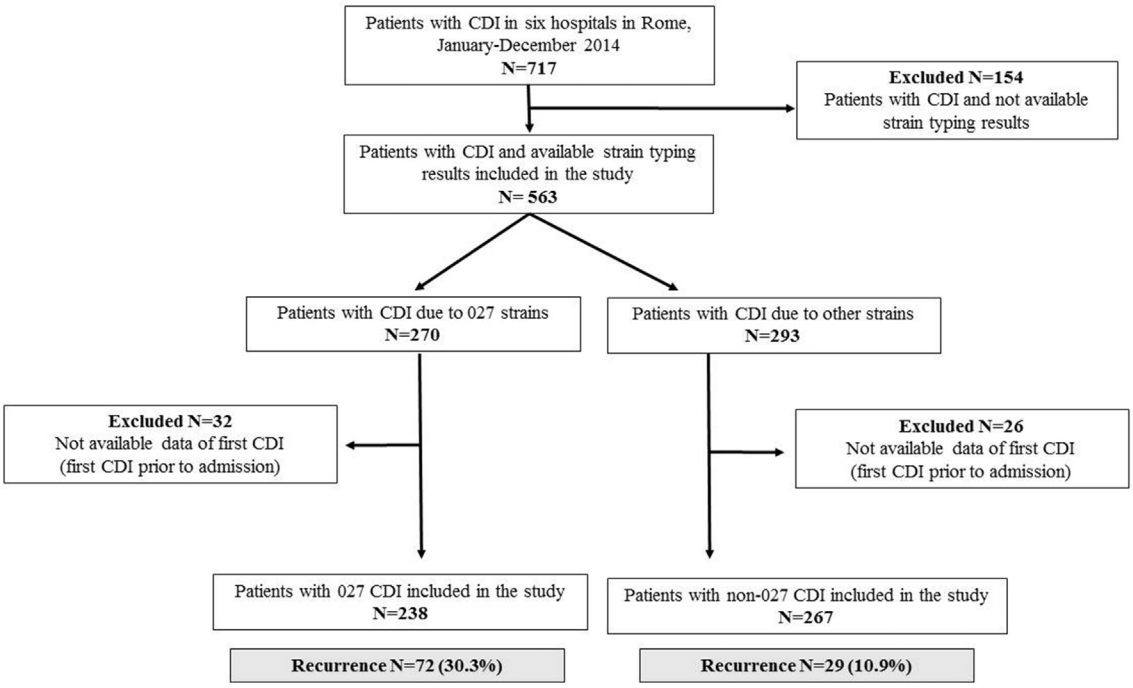

Fig. 1. Study flow chart. CDI, *Clostridium difficile* infection.

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Table 1
Comparison of baseline characteristics, disease severity, and treatment of patients with first infection due to 027 and non-027 *Clostridium difficile* strains

Characteristic	027+ $n = 238$	027− $n = 267$	p value
Demographics:			
Male sex	128 (53.8%)	117 (43.8%)	**0.025**
Age in years (IQR)	72 (65–81)	72 (65–81)	0.809
Ward of hospitalization:			
Internal Medicine	206 (86.6%)	246 (92.1%)	**0.041**
Surgery	26 (10.9%)	19 (7.1%)	0.134
ICU	6 (2.5%)	2 (0.7%)	0.111
Place of acquisition:			
Community-associated	24 (10.1%)	17 (6.4%)	0.127
Healthcare-associated			
Community onset healthcare-associated	26 (10.9%)	42 (15.7%)	0.114
Hospital onset	114 (47.9%)	159 (59.6%)	**0.009**
Nursing-home onset	74 (31.1%)	49 (18.4%)	**0.001**
Charlson comorbidity index:			
Median (IQR)	7 (3–7)	7 (3–7)	0.912
Other ongoing conditions:			
Diabetes	130 (54.6%)	137 (51.3%)	0.457
Cardiovascular disease	141 (59.2%)	155 (58.1%)	0.786
Chronic renal failure	105 (44.1%)	107 (40.1%)	0.358
COPD	117 (49.2%)	123 (46.1%)	0.487
Liver disease	10 (4.2%)	21 (7.9%)	0.087
IBD	4 (1.7%)	13 (4.9%)	**0.047**
Medications during 14 days prior to infection:			
Any immunusuppressive treatment	101 (42.4%)	104 (38.9%)	0.426
Any antibiotic	124 (46.4%)	207 (77.5%)	0.866
Medication during the CDI episodes:			
Concomitant use of PPI	194 (81.5%)	222 (83.1%)	0.631
Concomitant antibiotic therapy	92 (38.7%)	124 (46.4%)	0.077
Severe CDI[a]	124 (52.1%)	97 (36.3%)	**<0.001**
Treatment of first CDI episode:			
Vancomycin monotherapy	133 (55.9%)	194 (72.7%)	**<0.001**
Metronidazole monotherapy	66 (27.7%)	38 (14.2%)	**<0.001**
Vancomycin + metronidazole	39 (16.4%)	35 (16.1%)	0.298
Vancomycin >500 mg/day	110 (46.2%)	56 (21%)	**<0.001**
Recurrence	72 (30.3%)	29 (10.9%)	**<0.001**
14-day mortality	20 (8.4%)	8 (3%)	**0.008**
30-day mortality	48 (20.2%)	15 (5.6%)	**<0.001**

Statistical significant variables ($p \leq 0.05$) were highlighted in bold. CDI, *Clostridium difficile* infection; COPD, chronic obstructive pulmonary disease; IBD, inflammatory bowel disease; ICU, intensive care unit; IQR, interquartile range; PPI, protonic pump inhibitors.

[a] Severe CDI disease was defined as development of ileus, toxic megacolon, or pseudomembranous colitis within 5 days of the positive *C. difficile* stool specimen, or unexplained serum white blood count ≥15,000 cells/mm^3, or a serum creatinine level ≥1.5 times the premorbid level within 1 calendar day of collection of the stool specimen.

Table 5 shows multivariate analyses of factors independently associated with recurrence in the 027+ group, according to disease severity. Metronidazole monotherapy was an independent risk factor for recurrence in both mild/moderate CDI (sHR 1.894, 95%CI 1.052–3.410, p 0.033) and severe CDI (sHR 2.476, 95%CI 1.281–4.790, p 0.007).

Discussion

To our knowledge this is the first study evaluating risk factors for recurrence among patients with CDI due to 027 and non-027 strains in a large cohort of patients. The key message of our study is that metronidazole monotherapy is associated with a high risk of recurrence in patients with the first episode of CDI caused by the 027 strain, in both mild to moderate and severe cases. Instead, the initial choice of therapy (metronidazole or vancomycin) was not significantly associated with recurrence of CDI caused by non-027 strains.

Together with vancomycin, metronidazole has been considered the cornerstone of antibiotic treatment for CDI [19,20]. From the early 1980s, vancomycin became the drug of choice for treating CDI, especially because of its poor absorption from the intestinal tract [24]. Subsequently, with increasing concern for the development of vancomycin-resistant enterococci with oral vancomycin, the use of metronidazole quickly increased. Furthermore, several trials found that metronidazole and vancomycin are equally effective for the treatment of mild CDI, but that vancomycin is superior for treating patients with severe CDI [25,26]. Recent updates of treatment algorithms for CDI reconsidered the use of metronidazole as first-line therapy since it appears to be less efficacious than vancomycin in inducing clinical cure, especially for severe infections [27]. Based on these observations, current guidelines from the Infectious Diseases Society of America revised treatment recommendations for CDI and stated that either vancomycin or fidaxomicin is recommended over metronidazole for an initial episode of CDI [18]. Oral metronidazole is now recommended in settings where access to vancomycin or fidaxomicin is limited, for an initial episode of non-severe CDI only, while the combination of oral vancomycin and intravenous metronidazole should be used in cases of fulminant CDI, particularly if ileus is present [18]. Other studies have reported a lower incidence of recurrence in patients with 027− CDI treated with a prolonged and pulsed oral vancomycin regimen [28], and the emergence of some *Clostridium difficile* isolates (other than 027 strains) showing reduced susceptibility to metronidazole has been described [29]. Accordingly, with these recent recommendations, our results suggest that the use of metronidazole as first-line therapy should be discouraged in all cases of presumptive or documented CDI caused by a 027 ribotype, independently of disease severity.

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Table 2
Comparison of patients in whom infection recurred or not after the first *Clostridium difficile* infection, stratified for presumed ribotype

Characteristic	027+ ($n = 238$)			027− ($n = 267$)		
	First recurrence $n = 72$ (%)	Cure $n = 166$ (%)	p value	First recurrence $n = 29$ (%)	Cure $n = 238$ (%)	p value
Demographics:						
Male sex	41 (56.9%)	87 (52.4%)	0.519	14 (48.3%)	103 (43.3%)	0.609
Age (years): median (IQR)	72 (65–82)	72 (65–81)	0.651	72 (65–72)	72 (65–82.5)	0.088
Ward of hospitalization:						
Internal medicine	62 (86.1%)	144 (86.7%)	0.895	22 (75.9%)	224 (94.1%)	**0.001**
Surgery	10 (13.9%)	16 (9.6%)	0.334	5 (17.2%)	14 (5.9%)	**0.025**
ICU	0	6 (3.6%)	0.102	2 (6.9%)	0	**<0.001**
Place of acquisition:						
Community-associated	9 (12.5%)	15 (9%)	0.415	1 (3.4%)	16 (6.7%)	0.495
Healthcare-associated						
Community onset healthcare-associated	3 (4.2%)	23 (13.9%)	**0.028**	11 (37.9%)	31 (13%)	**0.001**
Hospital onset	19 (26.4%)	95 (57.2%)	**<0.001**	12 (41.4%)	147 (61.8%)	**0.035**
Nursing-home onset	41 (56.9%)	33 (19.9%)	**<0.001**	5 (17.2%)	44 (18.5%)	0.870
Charlson comorbidity index:						
Median (IQR)	7 (6–7)	5 (2–7)	**<0.001**	7 (3–7)	7 (2–7)	0.901
Other ongoing conditions:						
Diabetes	57 (79.2%)	73 (44%)	**<0.001**	17 (58.6%)	120 (50.4%)	0.404
Cardiovascular disease	45 (62.5%)	96 (57.8%)	0.501	13 (44.8%)	142 (59.7%)	0.126
Chronic renal failure	46 (63.9%)	59 (35.5%)	**<0.001**	1 (3.4%)	106 (44.5%)	**<0.001**
COPD	48 (66.7%)	69 (41.6%)	**<0.001**	14 (48.3%)	109 (45.8%)	0.800
Liver disease	3 (4.2%)	7 (4.2%)	0.986	0	21 (8.8%)	0.096
IBD	1 (1.4%)	3 (1.8%)	0.818	0	13 (5.5%)	0.197
Medications during 14 days prior to infection:						
Any immunosuppressive treatment	52 (72.2%)	49 (29.5%)	**<0.001**	6 (20.7%)	98 (41.2%)	**0.033**
Any antibiotic	56 (77.8%)	130 (78.3%)	0.927	26 (89.7%)	181 (76.1%)	0.097
Medication during the CDI episodes:						
Concomitant use of PPI	62 (86.1%)	132 (79.5%)	0.229	24 (82.8%)	198 (83.2%)	0.953
Concomitant antibiotic therapy	31 (43.1%)	61 (36.7%)	0.359	12 (41.4%)	112 (47.1%)	0.563
Severe CDI[a]	34 (47.2%)	90 (54.2%)	0.321	5 (17.2%)	92 (38.7%)	**0.024**
Treatment of first CDI episode:			**<0.001**			**0.038**
Vancomycin monotherapy	35 (48.6%)	98 (59%)		22 (75.9%)	172 (72.3%)	
Metronidazole monotherapy	34 (47.2%)	32 (19.3%)		7 (24.1%)	31 (13%)	
Vancomycin + metronidazole	3 (4.2%)	36 (21.7%)		0	35 (14.7%)	
Vancomycin >500 mg/day	29 (40.3%)	81 (48.8%)	0.226	4 (13.8%)	52 (21.8%)	0.314

Statistical significant variables ($p \leq 0.05$) were highlighted in bold. CDI, *Clostridium difficile* infection; COPD, chronic obstructive pulmonary disease; IBD, inflammatory bowel disease; ICU, intensive care unit; IQR, interquartile range; PPI, protonic pump inhibitors.

[a] Severe CDI disease was defined as development of ileus, toxic megacolon, or pseudomembranous colitis within 5 days of the positive *C. difficile* stool specimen, or unexplained serum white blood count ≥15,000 cells/mm^3, or a serum creatinine level ≥1.5 times the premorbid level within 1 calendar day of collection of the stool specimen.

Table 3
Multivariate analysis of risk factors for recurrence among patients with 027 *Clostridium difficile* infection (CDI)

	Multivariate analysis			
	sHR	95.0%CI		p value
		Lower	Upper	
Therapy for first CDI (*vancomycin as reference variable*):				
Metronidazole monotherapy versus vancomycin monotherapy	2.380	1.549	3.650	**<0.001**
Metronidazole + vancomycin versus vancomycin monotherapy	0.349	0.105	1.150	0.084
Any immunosuppressive therapy	3.116	1.906	5.090	**<0.001**

Statistical significant variables ($p \leq 0.05$) were highlighted in bold. sHR, subdistributional hazard ratio.

Table 4
Multivariate analysis of risk factors for recurrence among patients with *Clostridium difficile* infection (CDI) due to non-027 strains

	Multivariate analysis			
	sHR	95.0%CI		p value
		Lower	Upper	
Therapy for first CDI *(vancomycin as reference variable)*:				
Metronidazole monotherapy versus vancomycin monotherapy	1.896	0.974	3.690	0.060
Metronidazole + vancomycin versus vancomycin monotherapy	–	–	–	–
Non-severe CDI	3.704	1.437	9.524	**0.007**
Absence of chronic renal failure	16.129	2.155	125.000	**0.007**

Statistical significant variables ($p \leq 0.05$) were highlighted in bold. sHR, subdistributional hazard ratio.

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Table 5
Multivariate analysis of factors associated with recurrence in patients with presumed 027 *Clostridium difficile* infection (CDI) according to disease severity

	Mild/moderate disease (n=114)				Severe disease (n=124)			
	sHR	95.0%CI		p	sHR	95.0%CI		p value
		Lower	Upper			Lower	Upper	
Any immunusuppressive treatment	2.375	1.294	4.360	**0.005**	1.879	1.183	19.640	**0.008**
Treatment of first CDI:								
Vancomycin monotherapy	*Ref*[a]	*Ref*[a]	*Ref*[a]	**0.033**	*Ref*[a]	*Ref*[a]	*Ref*[a]	**0.007**
Metronidazole monotherapy	1.894	1.051	3.410	0.210	2.476	1.281	4.790	0.250
Vancomycin + metronidazole	0.378	0.083	1.730		0.302	0.040	2.280	

Statistical significant variables ($p \leq 0.05$) were highlighted in bold. sHR, subdistributional hazard ratio.
[a] All variables have been tested versus vancomycin (vancomycin monotherapy as reference variable).

Of importance, a very low risk of recurrence has been observed in patients treated with combination therapy (vancomycin plus metronidazole). However, probably due to the small number of patients in this category, combination therapy was not a protective factor for recurrence at multivariate analysis in both 027+ and 027− groups. Thus, the strategy of a combination therapy (adopted more frequently in two study centres, Policlinico Torvergata and Policlinico Gemelli) should be considered, especially in more compromised patients [18].

Our study has some strengths and several limitations. The potential limitations are the following. (a) It is an observational study. (b) Presumptive ribotyping was performed in the majority (about 80%) but not all CDI cases. (c) the RT-PCR method used (Xpert *C. difficile* Epi assay; Cepheid) allows a presumptive identification of ribotype; the standard toxigenic cultures were not clinically practical because of slow turnaround times, and it has been reported that Xpert, *C.difficile* Epi displays sensitivities and specificities of 96.6–99.7% and 93.0–98.6%, respectively [30,31]. (d) Since the isolates from recurrence were not re-tested by Xpert, *C.difficile* Epi or other methods, we were not able to distinguish with certainty a recurrence from a reinfection. (e) All cases were collected in central Italy, a region of high prevalence of the 027 strain [32]. (f) The adjusted HR for metronidazole monotherapy and recurrence in the 027− patient group approached statistical significance, but due to the small numbers of patients we were not able to perform a subgroup analysis of severe and non-severe CDI in this patient group.

The strengths of our study are its multicentre design, the large sample size, the accuracy of data collection, and the fact that this is the largest study to date to evaluate risk factors for recurrence among the specific population of 027− CDI in a real-world setting. Furthermore, all cases were collected in the absence of a recognized epidemic outbreak.

We conclude that patients affected by 027+ CDI have some peculiar risk factors for recurrence. Compared to vancomycin, metronidazole monotherapy is a major risk factor for recurrence in patients with CDI due to the 027 strain, independently of disease severity. Thus, vancomycin instead of metronidazole seems to be the drug of choice in all patients with a first episode of CDI, especially in those with infection due to the 027 strain. Based on these observations, we suggest that ribotyping to identify 027 or non-027 strains should be performed in clinical practice to guide therapy.

Transparency declaration

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.cmi.2018.06.020.

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