

Role of binary toxin in the outcome of *Clostridium difficile* infection in a non-027 ribotype setting

E. REIGADAS^{1,2,3*}, L. ALCALÁ^{1,3,4}, M. MARÍN^{1,2,3,4}, A. MARTÍN^{1,3},
C. IGLESIAS^{1,3,4} AND E. BOUZA^{1,2,3,4*}

¹ Department of Clinical Microbiology and Infectious Diseases, Hospital General Universitario Gregorio Marañón, Madrid, Spain

² Medicine Department, School of Medicine, Universidad Complutense de Madrid (UCM), Madrid, Spain

³ Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain

⁴ CIBER de Enfermedades Respiratorias (CIBERES CB06/06/0058), Madrid, Spain

Received 1 September 2014; Final revision 6 April 2015; Accepted 11 June 2015;
first published online 29 June 2015

SUMMARY

Binary toxin (BT) has been associated with strains causing more severe *Clostridium difficile* infection (CDI), such as ribotype 027. Data on the outcome of patients having BT present in ribotypes other than 027 are scarce. Our objective was to investigate the association between BT isolates and outcome of CDI in a non-027 ribotype setting. We prospectively included CDI episodes (January–June 2013 and March–June 2014) from symptomatic patients aged >2 years. Epidemiological and clinical data were recorded. BT genes were detected using multiplex PCR. During the study period, we identified 326 episodes of CDI, of which 319 were available for molecular analysis. Of these, 54 (16.9%) were caused by *C. difficile* strains with BT. Most (90.7%) isolates with BT were ribotype 078/126. CDI patients with BT-positive strains did not differ from those with BT-negative strains in terms of recurrence (13.0% vs. 15.5%, $P = 0.835$), treatment failure (0.0% vs. 2.3%, $P = 0.594$), overall mortality (11.1% vs. 9.1%, $P = 0.612$), or CDI-related mortality (0.0% vs. 1.9%, $P = 0.612$). Multivariate regression revealed no association between BT and poor outcome. In conclusion, in a non-027 setting, we found that most BT isolates were 078/126 and were not associated with poor outcome.

Key words: Binary toxin, *Clostridium difficile* infection, outcome, recurrence, ribotype, severe.

INTRODUCTION

Clostridium difficile infection (CDI) is the leading cause of hospital-acquired diarrhoea and is associated

with a considerable health and economic burden [1–4]. Between 12% and 18% of patients progress to severe disease [5–7], and about 20% develop ≥ 1 recurrent episodes (poor outcome) [8]. Although several host-related and pathogen-related factors have been proposed as risk factors for poor outcome of CDI, the reasons why some patients experience severe disease and poor outcome remain unclear [9].

The virulence of *C. difficile* is caused by two major toxins (toxin A and toxin B). Some *C. difficile* ribotypes, including ribotype 027 strains, also produce a binary toxin (BT) [10, 11]. In the past decade,

* Author for correspondence: E. Reigadas, PharmD, Servicio de Microbiología Clínica y Enfermedades Infecciosas, Hospital General Universitario ‘Gregorio Marañón’, C/ Dr. Esquerdo, 46, 28007 Madrid, Spain.

(Email: helenrei@hotmail.com) [E.R.]

(Email: emilio.bouza@gmail.com) [E.B.]

Some of the data in this study were presented previously in poster form at the International Congress of Antimicrobial Agents and Chemotherapy, 9 September 2014, Washington, DC, USA.

C. difficile ribotype 027/NAP1/BI and its BT have been associated with poor outcome [12].

However, the role of BT in the outcome of CDI episodes caused by ribotypes other than 027/NAP1/BI has rarely been explored.

Our objective was to investigate the role of BT isolates in the severity and outcome of CDI in a non-027 ribotype setting.

MATERIAL AND METHODS

Design and study population

From January 2013 to June 2013, we prospectively included all patients diagnosed with CDI in our institution. Children aged <2 years and patients with recurrence of an episode from before the study period were not included. Patients were followed-up throughout the study period and for at least 2 months after their last CDI episode or recurrence.

In our institution, the presence or absence of BT is not routinely reported to the patient's physician nor was it reported during the study period.

Definitions

A CDI episode was defined as a positive test result for toxigenic *C. difficile* and the presence of diarrhoea (≥ 3 unformed stools in 24 h) or colonoscopic findings demonstrating pseudomembranous colitis.

The type of CDI episode according to the potential site of acquisition was defined according to the criteria of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for *Clostridium difficile* [13].

Severity of CDI was defined according the guidelines of the Society of Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) for mild to moderate and severe CDI [14]. The definition of severe complicated CDI was slightly modified, as follows: CDI in patients with septic shock or colectomy or megacolon or CDI-related admission to the intensive care unit (ICU) within 7 days of the positive sample or CDI-attributable mortality.

Recurrent CDI (R-CDI) was defined as the return of symptoms after recovery from a previous episode (at least 3 days without diarrhoea and clinical improvement) and a positive stool sample separated from the previous sample by 15–60 days. Episodes occurring >60 days after the previous episode were not considered recurrences but new episodes.

Treatment was considered to have failed when the patient did not recover from a CDI episode (≥ 3 loose bowel movements or stool consistency did not improve and persistence of fever, abdominal pain or analytical parameters of disease severity did not improve) and a stool sample separated from the previous sample by <15 days was positive.

Poor outcome was defined as R-CDI, treatment failure, or progression to severe complicated CDI.

Mortality was considered to be associated with CDI when death was not clearly attributable to other unrelated causes, when it occurred within 10 days of the CDI diagnosis, or when it was due to well-known complications of CDI.

Data collection

The demographic data collected included age, sex, hospital department or outpatient clinic at the time of diagnosis, and history of hospital admissions up to 3 months prior to infection (to determine the source of CDI). Data regarding the underlying condition were recorded using the McCabe & Jackson classification, and comorbidity was assessed according to the Charlson index [15, 16]. Risk factors for CDI during the month prior to the diagnosis of CDI were recorded.

The data recorded for the CDI episode were as follows: days of diarrhoea, presence of abdominal pain, abdominal distension, fever, hypotension, toxic megacolon, pseudomembranous colitis, and severity of CDI. Antibiotic treatment for the CDI episode and outcome (need for ICU admission, need for surgery for the CDI episode, recurrence, mortality, and CDI-associated mortality) were also recorded.

Laboratory procedure

All samples underwent toxigenic culture in *Clostridium* selective agar medium (bioMérieux, France); plates were incubated under anaerobic conditions at 35–37 °C for 48 h. Following incubation, colony morphotypes compatible with *C. difficile* were selected with the help of a binocular magnifying glass. Colonies suspected of being toxigenic *C. difficile* were finally identified using the immunochromatographic system (C Diff Quik-Chek Complete assay, TechLab, USA) and the MRC-5 cell line cytotoxicity test.

The genes of toxin A (tcdA conserved fragment, tcdA deleted fragment), toxin B (tcdB), and BT (cdtA and cdtB) were detected using multiplex

polymerase chain reaction (PCR) with an internal amplification control following a method adapted from other studies [17, 18].

All the toxigenic strains (presence of any of the genes of the toxins) were characterized using PCR ribotyping according to the procedure described by Stubbs *et al.* [19]. Phylogenetic analysis of ribotyping profiles was performed using the unweighted pair-group method with arithmetic mean (UPGMA) and Dice coefficients (Bionumerics v. 5.0, Applied Maths, Belgium). The profiles of our isolates were compared with international ribotyping profile libraries. Ribotypes were named using the international designation. When the correspondence with international ribotypes was unknown, the letter R followed by a number was used.

Data analysis

All analyses were performed using SPSS v. 18.0 (SPSS Inc., USA). Qualitative variables appear with their frequency distribution. Quantitative variables are expressed as the median and interquartile range (IQR). Groups were compared using Fisher's exact test for categorical variables and the *t* test (normal distribution) or Mann–Whitney test (non-normal distribution) for continuous variables. In order to determine the risk factors for poor outcome, we performed a bivariate analysis between the variables collected and poor outcome. All variables with $P < 0.05$ and the BT variable were included in a multivariate logistic regression model to assess their correlation with poor outcome; we also performed separate analyses for R-CDI, mortality, and CDI-related mortality. The odds ratio (OR) and 95% confidence interval (CI) were calculated. A P value < 0.05 was considered significant.

Ethical issues

This study was approved by the Ethics Committee of Hospital General Universitario Gregorio Marañón and the Spanish Agency for Medicines and Health Care Products.

RESULTS

During the study period, 326 CDI episodes met our inclusion criteria. Of these, 319 episodes were available for molecular analysis of the strains. We detected 54 (16.9%) episodes caused by *C. difficile* strains with

a BT gene. Six of the 54 episodes involved a co-existing strain which lacked the BT gene.

Out of the 54 episodes caused by strains with BT, 49 (90.7%) were ribotype 078/126, in three (5.6%) episodes there was no correspondence with international ribotypes and two (3.7%) were ribotype 023.

The demographic and clinical characteristics of CDI episodes are described in Table 1. Of the 319 CDI episodes, 82.4% were mild to moderate and 17.6% were considered severe to severe complicated. Outcome was poor in 63 (19.7%) episodes; of these, 48 (15.0%) were R-CDI, six (1.9%) were considered treatment failures, and nine (2.8%) progressed to severe complicated disease. Pseudomembranous colitis was observed in four patients. Overall mortality was 9.4%, and mortality attributable to CDI was 1.6%.

Epidemiological and baseline characteristics of patients with CDI caused by BT-positive or BT-negative strains (Table 1)

We found no differences between patients with CDI caused by a BT-positive strain and those with a BT-negative strain regarding age, sex, type of underlying condition or Charlson comorbidity index score. We did not find any significant differences in risk factors between the groups.

Regarding the CDI episode, no differences were found in the potential site of acquisition (community or healthcare setting). In both groups, most of the CDI episodes were mild to moderate (75.9% BT positive vs. 83.8% BT negative, $P = 0.292$), there were no significant differences in CDI severity between BT-positive and BT-negative episodes.

Comparison of outcome between patients with CDI caused by BT-positive or BT-negative strains (Table 2)

Patients with BT-positive strains presented a median of 1 more day of diarrhoea (BT-positive median 4.0 days of diarrhoea vs. BT-negative median 3.0 days, $P = 0.198$); however, it was not statistically significant. Patients with BT-positive strains had a shorter overall hospital stay than patients with BT-negative strains (BT-positive median 14.0 overall days stay vs. BT-negative median 19.0 days, $P = 0.012$); however, there were no significant differences in days of hospital stay after the diagnosis of the CDI episode ($P = 0.384$).

We found no differences between BT-positive and BT-negative CDI episodes in terms of recurrence

Table 1. Epidemiological and baseline clinical characteristics of CDI patients with and without BT

Characteristics	Overall (N = 319)	BT positive (n = 54)	BT negative (n = 265)	P value
Demographic data				
Male gender, n (%)	159 (49.8)	29 (53.7)	130 (49.1)	0.554
Age, years, median (IQR), McCabe & Jackson, n (%)	71.3 (49.8–82.2)	74.5 (58.1–82.9)	69.3 (47.9–82.1)	0.204
Non-fatal	282 (88.4)	45 (83.3)	237 (89.4)	0.241
Ultimately fatal	31 (9.7)	7 (13.0)	24 (9.1)	0.447
Rapidly fatal	5 (1.6)	2 (3.7)	3 (1.1)	0.200
Charlson index score, median (IQR)	2.0 (0.0–4.0)	2.0 (1.0–4.0)	2.0 (0.0–4.0)	0.819
Risk factors, n (%)				
Previous antibiotic	263 (82.7)	47 (87.0)	216 (81.8)	0.433
Proton pump inhibitors	242 (76.3)	45 (83.3)	197 (74.9)	0.220
Nasogastric tube	32 (10.1)	5 (9.3)	27 (10.2)	1.000
Irritable bowel disease	21 (8.5)	6 (11.1)	21 (8.0)	0.427
Surgery	53 (16.7)	6 (11.1)	47 (17.8)	0.316
Mechanical ventilation	32 (10.1)	3 (5.6)	29 (11.0)	0.321
Chemotherapy	40 (12.5)	9 (16.7)	31 (11.7)	0.366
Dialysis	22 (6.9)	1 (1.9)	21 (7.9)	0.143
CDI episode, n (%)				
Healthcare-acquired CDI	230 (72.1)	35 (64.8)	195 (73.6)	0.243
Community-acquired CDI	68 (21.3)	14 (25.9)	54 (20.4)	0.366
Indeterminate-CDI	21 (6.6)	5 (9.3)	16 (6.0)	0.371
Severity of CDI episode, n (%)				
Mild to moderate	263 (82.4)	41 (75.9)	222 (83.8)	0.173
Severe to severe complicated	56 (17.6)	13 (24.1)	43 (16.2)	0.173

BT, Binary toxin; CDI, *Clostridium difficile* infection; IQR, interquartile range.

Table 2. Outcome of CDI patients with and without BT

Characteristics	Overall (N = 319)	BT positive (n = 54)	BT negative (n = 265)	P value
Days with diarrhoea, median (IQR)	3.0 (2.0–6.0)	4.0 (2.0–6.25)	3.0 (2.0–5.25)	0.198
Overall hospital stay, days, median (IQR)	17.0 (11.0–37.0)	14.0 (8.0–25.75)	19.0 (12.0–41.5)	0.012
Hospital stay, days, after diagnosis of CDI, median (IQR)	8.0 (4.0–15.0)	6.0 (4.5–13.0)	8.0 (4.0–19.75)	0.384
Recurrence, n (%)	48 (15.0)	7 (13.0)	41 (15.5)	0.835
Treatment failures, n (%)	6 (1.9)	0 (0.0)	6 (2.3)	0.594
Overall mortality, n (%)	30 (9.4)	6 (11.1)	24 (9.1)	0.612
CDI-related mortality, n (%)	5 (1.6)	0 (0.0)	5 (1.9)	0.553

BT, Binary toxin; CDI, *Clostridium difficile* infection; IQR, interquartile range.

rate (13.0% vs. 15.5%, $P = 0.835$), overall mortality (11.1% vs. 9.1%, $P = 0.612$), or CDI-related mortality (0% vs. 1.9%, $P = 0.553$).

In order to determine the risk factors for poor outcome, we performed a multivariate logistic regression model which included BT as a variable to assess its correlation with poor outcome. There was no association between BT and poor outcome (OR 0.793, 95% CI 0.243–2.591, $P = 0.701$).

We also performed separate analyses for R-CDI, mortality, and CDI-related mortality. None of these

analyses revealed any association with the presence of BT ($P > 0.05$).

DISCUSSION

In our institution, CDI episodes caused by strains harbouring BT genes accounted for 16.9% of the CDI episodes. None of the strains were ribotype 027; most strains with BT were ribotype 078/126. We found that the presence of BT genes was not associated with poor outcome.

Since the first description of BT by Popoff *et al.* [20] in 1988, the prevalence of BT-positive strains has been relatively low (6.4%) [21]. However, the outbreaks caused by *C. difficile* ribotype 027/NAP1/BI in Europe, Canada, and the United States revealed an increase in prevalence, which reached as high as 82% of all clinical isolates during the outbreaks [12, 22, 23]. In our study, the prevalence of BT-positive strains was similar to that reported for settings with a low prevalence of ribotype 027 [11, 24, 25]. None of the strains belonged to ribotype 027, this is in line with low prevalence of ribotype 027 in our country (Spain), which has been reported to range between 0% and 2.9% in two prevalence national studies and a European multicentre study [25–27].

The predominant ribotype of BT-positive strains was ribotype 078/126, which has been reported to be a ‘hypervirulent strain’ [28]. Nowadays, the association between outcome and specific ribotype is open to debate, and studies of the association with the so-called hypervirulent ribotypes 078 and 027 in non-epidemic settings revealed no association between specific ribotypes and disease severity [29, 30].

An association between disease severity and BT was described in two retrospective studies by Barbut *et al.* [24, 31]; however, in both studies the number of patients included that were positive for BT was very low (14 and 26 patients). We found no significant association between disease severity and BT. Another recent prospective study also failed to demonstrate a statistically significant association between disease severity and BT [32].

The presence of the BT gene was linked to an increased risk of R-CDI in a recent study [33], interestingly in this study, ribotype 027 was not associated to R-CDI while ribotype 078 was found to be associated with R-CDI [33]. We found that the frequency of recurrence did not vary in BT-positive and BT-negative CDI patients. Other recent studies have also observed this lack of association between presence of BT and recurrence [32, 34].

In a retrospective registry-based study, higher case fatality was observed for patients with a BT-positive strain than for those with a BT-negative strain [35]. However, in this study, the authors were not able to obtain information on the cause of death, therefore CDI-related mortality and its association with the BT could not be examined. In our study, we found no differences between overall mortality of BT-positive and BT-negative CDI episodes. In fact, all cases of CDI-related mortality were recorded in

BT-negative CDI patients. This finding is in line with the results published by Kim *et al.* [32], in which they observed no association between overall mortality or CDI-associated mortality.

Our study is limited by the fact that it was performed in a single centre, therefore our data cannot necessarily be extrapolated to other centres; a further limitation is that most of the BT strains corresponded to ribotype 078. In addition, we were not able to measure the actual production of BT.

In conclusion, in a non-027 setting, the prevalence of BT is significant. We found no association between recurrence of infection caused by BT strains, progress of disease severity, or mortality (poor outcome). It is possible that the association between BT and poor outcome has been overestimated, since it is based on data from the 027/NAP1/BI outbreaks.

ACKNOWLEDGEMENTS

We thank Thomas O’Boyle for his help in the preparation of the manuscript. This study was partially financed by Astellas Pharma Inc., the Rafael del Pino Foundation, and Fondo de Investigaciones Sanitarias (FIS), Research Project number PI13/00687. Elena Reigadas holds a grant from the Río Hortega programme of the Carlos III Health Institute, Spanish Government.

DECLARATION OF INTEREST

None.

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