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Risk Factors and Outcomes for Bloodstream Infections Secondary to *Clostridium difficile* Infection

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We determined the incidence, risk factors, and outcomes of bloodstream infections (BSI) subsequent to *Clostridium difficile* infection (CDI). We performed a retrospective study of all patients with definite diagnosis of CDI admitted from January 2014 to December 2014 in two large hospitals in Rome. Two groups of patients were analyzed: those with CDI and subsequent BSI (CDI/BSI$^+$) and those with CDI and no evidence of primary BSI (CDI/BSI$^-$). Data about clinical features, microbiology, treatments, and mortality were obtained. Overall, 393 cases of CDI were included in the final analysis: 72 developed a primary nosocomial BSI, while 321 had CDI without microbiological and clinical evidence of BSI. Etiologic agents of BSI were *Candida* species (47.3%), *Enterobacteriaceae* (19.4%), enterococci (13.9%), and mixed infections (19.4%). In multivariate analysis, ribotype 027 status (odds ratio [OR], 6.5), CDI recurrence (OR, 5.5), severe CDI infection (OR, 8.3), and oral vancomycin at >500 mg/day (OR, 3.1) were recognized as factors independently associated with the development of nosocomial BSI. Thirty-day mortality from CDI diagnosis was higher for patients of the CDI/BSI$^+$ group than for the controls (38.9 versus 13.1%; $P < 0.001$). Among patients of the CDI/BSI$^+$ group, mortality attributable to primary BSI was as high as 57%. Our findings suggest that severe CDI is complicated by the development of nosocomial BSI. *Candida* species and enteric bacteria appear to be the leading causative pathogens and are associated with poor outcomes.

Clostridium difficile infection (CDI) is an emerging infection, usually occurring after exposure to broad-spectrum antibiotics (1–3). This infection can be mild and self-limiting but might progress to severe disease with ileus, toxic megacolon, and eventually, death. The incidence, severity, and acquisition of infection of people formerly classified as being at low risk seem to be increasing, and a hypervirulent, fluoroquinolone-resistant *C. difficile* strain, named NAP1/BI/027, is associated with severe symptoms, high recurrence rates, and poor outcomes (4–6).

The alterations occurring in the intestinal flora, which is recognized as a microbiome, may promote the translocation of pathogens in the blood and the development of nosocomial bloodstream infections (BSIs) (7). Recently, we reported our experience of studying candidemia subsequent to severe CDI (8–10), and we observed an association between *Candida* species BSI and CDI, especially if caused by ribotype 027 strains. We reported a case of severe community-onset health care-associated CDI complicated by carbapenemase-producing *Klebsiella pneumoniae* BSI (11). Thus, it was hypothesized that antibiotic therapy and/or other clinical characteristics related to CDI (i.e., severity, recurrence, disease caused by a highly virulent strain, etc.) contribute to alterations of the colon indigenous microbiota and eventually predispose patients to BSI (12–14).

The aim of our study was to analyze the clinical findings for patients with CDI and primary nosocomial BSI to determine the risk factors and outcomes associated with these infections.

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MATERIALS AND METHODS

Study design and study patients. This was a retrospective study of patients who were admitted from January 2014 to December 2014 to two large hospitals in Rome: Policlinico Umberto I-Sapienza University Hospital (1,200 beds and 49,000 admissions/year in 2014) and the San Giovanni-Addolorata Hospital (700 beds and 30,000 admissions/year in 2014). All adults (aged >18 years) with a documented CDI initially were included in the study. Patients for whom we could not obtain medical records were excluded from the final analysis. The ethics committee of the Policlinico Umberto I approved the study.

Data were extracted from the medical records of patients and from hospital computerized databases or clinical charts according to a prepared questionnaire. The following data were reviewed: demographics, clinical and laboratory findings, comorbidity conditions (like diabetes mellitus, cardiovascular disease, pulmonary disease, renal disease, hepatic disease, central nervous system disease, malignancy, and the overall number of comorbidity conditions), microbiological data, duration of hospital stay, incidence of infections during hospitalization, treatments and procedures during hospitalization and/or in the previous 90 days prior to infection (immunosuppressive therapy, placement of a central venous catheter [CVC] or a urinary catheter, dialysis, endoscopic procedures, tracheostomy, surgery, and mechanical ventilation), admission from a long-term-care facility or a nursing home, classes of antibiotics received on admission and/or after admission before a positive culture was obtained, the sequential organ failure assessment (SOFA) score at the time of infection, side effects, and 30-day mortality.

Data on antibiotic therapy in the previous 30 days as well as other risk factors for multidrug-resistant (MDR) organisms were derived from the following sources: (i) history taken from patients and/or relatives, (ii)

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discharge letters and summaries if patients were previously hospitalized in other facilities, and (iii) electronic charts if patients were previously hospitalized or seen in the clinics involved in the study.

Study definitions. CDI was defined as (i) the presence of diarrhea (i.e., passage of three or more unformed stools in 24 or fewer consecutive hours) and (ii) a stool test result positive for the presence of toxigenic *C. difficile* or its toxins or colonoscopic or histopathologic findings demonstrating pseudomembranous colitis (16). The same criteria were used to diagnose recurrent CDI. BSI was defined according to the standard definitions of the Centers for Disease Control and Prevention (CDC) (17). For common skin contaminants (i.e., diphtheroids, *Bacillus* spp. [not *B. anthracis*], *Propionibacterium* spp., coagulase-negative staphylococci [including *S. epidermidis*], viridans group streptococci, *Aerococcus* spp., and *Micrococcus* spp.), bacteremia was considered clinically significant if at least two blood cultures were positive and associated with at least two signs or symptoms of systemic inflammatory response (17). Candidemia was defined as the isolation of microorganisms in one or more separate blood cultures with clinical evidence of infection (18).

Severe sepsis was defined as sepsis with sepsis-induced organ dysfunction or tissue hypoperfusion (manifesting as hypotension, elevated lactate levels, or decreased urine output). Septic shock was defined as severe sepsis plus persistently low blood pressure following the administration of intravenous fluids (19). The CVC was considered the likely source of infection if blood culture obtained from the lumen of the catheter (but not from peripheral veins) was positive in a time of <2 h and/or if culture of samples from the catheter was positive (20). Primary BSI was defined as BSI occurring in patients without a recognized source of infection. Patients with secondary BSI (defined as BSI with a documented source of infection, including intravascular device, urinary tract infection, pneumonia, intra-abdominal infection, skin and soft-tissue infection, localized abscess, central nervous system infection, and/or infective endocarditis) were excluded from the final analysis.

BSI was defined as a nosocomial infection if it occurred more than 48 h after admission to the hospital and if no signs or symptoms of infection were noted at the time of hospital admission. *Candida* colonization at the time of admission was defined as a fungal species-positive culture from any of the tested surveillance sites, and colonization was considered unifocal or multifocal when *Candida* species were isolated from one focus or simultaneously from various noncontiguous foci, respectively (21).

Severe CDI was defined as a white blood cell count of $>15{,}000$ cells/μl or a serum creatinine level ≥ 1.5 times higher than the premorbid level (16). The amount of time at risk is a measure of the risk of developing some new condition within a specified period of time: time at risk for CDI was considered the median of the time between hospitalization and clinical development of CDI, time at risk for CDI recurrence was considered the median of the time between the first CDI and the recurrence of infection, and time at risk for BSI was considered the median of the time between CDI and BSI. MDR pathogens were defined according to standard definitions (22, 23). Data from all patients were entered in an electronic database.

Study groups, endpoints, and measurement of outcomes. All patients with definite diagnosis of CDI were divided in two groups: those developing a primary BSI within 30 days from initial diagnosis of CDI (CDI/BSI$^+$ group) and patients with CDI and no evidence of primary BSI (CDI/BSI$^-$ group). The obtainable records of all patients belonging to the CDI/BSI$^+$ and CDI/BSI$^-$ groups were compared.

The clinical endpoints of our study were the assessment of risk factors for the development of BSI and the evaluation of 30-day mortality (24) rates in both groups. All of the outcomes in the two groups were measured after the main acute event during hospitalization: the first CDI, the recurrence of CDI, and primary BSI (only in patients of the CDI/BSI$^+$ group).

Outcomes of primary BSI episodes were assessed as (i) cure, in the case of complete disappearance of clinical, radiological, and microbiological signs (i.e., repeated negative cultures) of infection at the time of hospital discharge, and (ii) attributable mortality, in the case of BSI-related death due to clinical evidence of infection at the time of death without an alternative cause of death or autopsy evidence of tissue infection (25).

TABLE 1 Etiologies of BSI

Pathogen(s)[a]	No. (%) CDI/BSI$^+$ ($n = 72$)
Enterobacteriaceae	14 (19.4)
Enterococcus species	10 (13.9)
Candida species	34 (47.3)
C. albicans	15 (44.1)
C. glabrata	9 (26.5)
C. tropicalis	5 (14.7)
C. parapsilosis	3 (8.9)
C. krusei	1 (2.9)
C. guilliermondii	1 (2.9)
Mixed BSI[b]	14 (19.4)
C. albicans-E. faecalis	6 (42.9)
E. faecalis-K. pneumoniae	3 (21.5)
C. glabrata-K. pneumoniae	2 (14.3)
C. tropicalis-K. pneumoniae	1 (7.1)
E. faecium-K. pneumoniae	1 (7.1)
C. tropicalis-E. faecium	1 (7.1)

[a] Multidrug-resistant pathogens include 8 strains of carbapenemase-producing *K. pneumoniae* and 7 strains of vancomycin-resistant enterococci. ESBL isolates included 6 strains of *K. pneumoniae*, 4 strains of *E. cloacae*, and 1 strain of *E. coli*.
[b] Isolation of bacteria plus fungi.

Microbiological analysis. Microbiological diagnosis of *C. difficile* disease was performed by using enzyme immunoassays (EIAs) combining the detection of *C. difficile* glutamate dehydrogenase (GDH) and toxin A/B antigens in stool specimens. We used the commercial methods TechLab C. Diff Quik Chek Complete, Meridian Bioscience Immunocard *C. difficile* GDH, and Vidas *C. difficile* toxin A/B according to the testing algorithms established at the individual study sites. Stool specimens also were tested by the Cepheid Xpert *C. difficile*/Epi assay, which is a multiplex real-time PCR that detects *tcdB*, the binary toxin gene (*cdt*), and the *tcdC* gene deletion at nucleotide 117 in order to identify the PCR ribotype 027 strain, also called strain 027/NAP1/BI (26).

To detect bacteria and/or fungi, blood specimens (from a peripheral venipuncture and/or intravascular catheter) were obtained for culture and processed using the automated Bactec system (Becton Dickinson Diagnostic Instruments, Sparks, MD). For bloodstream isolates, species identification was performed by micromorphology analysis and biochemical tests according to standard procedures.

Statistical analysis. All data were statistically analyzed using a commercially available statistical software package (SPSS, version 20.0; SPSS Inc., Chicago, IL). Continuous variables were compared using the Student *t* test for independent samples. Categorical variables were evaluated using the χ^2 test or Fisher exact test when appropriate. All tests were 2-tailed, and a P value of <0.05 was considered statistically significant. Results were expressed as means ± standard deviations (SD) for continuous normally distributed variables or as a percentage for categorical variables. Multivariate analysis was used to identify independent predictors of mortality and predictors of BSI. Matched bivariate analyses were conducted using a conditional logistic regression model, incorporating all variables found to be significant in univariate analysis ($P < 0.05$) with a stepwise method. The final model was tested for confounding factors (like the underlying severity of illness, comorbidities, and antibiotic use). If a covariate affected the ß-coefficient of a variable in the model by $>10\%$, then the confounding variable was maintained in the multivariable model.

TABLE 2 Clinical characteristics of patients with CDI/BSI compared to those of controls

Variable[a]	Value(s) by infection status		*P* value[b]
	CDI/BSI$^+$ ($n = 72$)	CDI/BSI$^-$ ($n = 321$)	
Age (yr)	74.4 ± 4.3	74.1 ± 5	0.8
Male sex (no. [%])	35 (48.6)	153 (47.6)	0.8
Presence of at least 2 comorbidities (no. [%])	72 (100)	295 (91.9)	0.07
COPD (no. [%])	30 (41.6)	121 (37.7)	0.2
Heart failure (no. [%])	27 (37.5)	101 (31.4)	0.08
Diabetes mellitus (no. [%])	31 (43)	124 (38.6)	0.07
Neoplasm (no. [%])	15 (20.8)	63 (19.6)	0.9
Chronic liver disease (no. [%])	7 (9.7)	37 (11.5)	0.8
Neurological disease (no. [%])	24 (33.3)	93 (28.9)	0.09
Immunosuppressive therapy (no. [%])			
Steroids	36 (50)	79 (24.6)	**0.001**
Chemotherapy	5 (6.9)	16 (5)	0.3
Chronic renal disease (no. [%])	29 (40.3)	123 (38.3)	0.8
IBD (no. [%])	7 (9.7)	15 (4.7)	0.05
Severe CDI infection (no. [%])	72 (100)	150 (46.7)	**<0.001**
CDI recurrence (no. [%])	60 (83.3)	95 (29.6)	**<0.001**
Number of recurrences > 1 (no. [%])	24 (33.3)	23 (7.1)	**<0.001**
Ribotype 027 (no. [%])	61 (84.7)	109 (33.9)	**<0.001**
Antibiotic therapy (previous 30 days) (no. [%])	62 (86.1)	275 (85.7)	0.1
Mean duration of previous antibiotic therapy (days)	10.3 ± 2.6	10.1 ± 3.1	0.6
Antifungal therapy (previous 30 days) (no. [%])	10 (13.9)	39 (12.1)	0.7
Mean duration of previous antifungal therapy (days)	6 ± 1.7	5.4 ± 1.3	0.1
Removable intravascular devices (no. [%])			
CVC	31 (43)	123 (40.5)	0.08
Pacemaker	9 (12.5)	38 (11.8)	0.7
Multifocal *Candida* colonization (no. [%])	6 (8.3)	15 (4.6)	**0.03**
Abdominal surgery (no. [%])	5 (6.9)	31 (9.6)	0.2
TPN (no. [%])	29 (40.3)	109 (33.9)	0.08
PPI therapy (no. [%])	72 (100)	321 (100)	1.0
Charlson comorbidity index (median)	3.7 ± 1.5	3.3 ± 1.6	0.1
SOFA score (median)	3.6 ± 0.8	1.7 ± 0.7	**<0.001**

[a] COPD, chronic obstructive pulmonary disease; IBD, inflammatory bowel disease; CVC, central venous catheter; MDR, multidrug resistant; TPN, total parenteral nutrition; PPI, proton pump inhibitors; SOFA, sequential organ failure assessment.
[b] Values in boldface are significant.

Confidence intervals (95% CI) were calculated. Multicollinearity was assessed according to the condition index of the multivariate model: a condition index of <10 denotes weak collinearity, 10 to 30 denotes moderate collinearity, and >30 denotes strong collinearity.

RESULTS

During the study period, 440 patients fulfilled criteria for CDI. Out of these, 19 patients with secondary BSI and 28 patients whose medical records were unobtainable were excluded from the final analysis. The final cohort of study comprised 393 patients. The incidence of CDI was 6.1 per 1,000 admissions to Policlinico Umberto I Hospital and 4.5 per 1,000 admissions to San Giovanni-Addolorata Hospital. The majority of patients with CDI were hospitalized in medical wards (63%), followed by intensive care unit (ICU) (18%) and surgery (19%) wards. Seventy-two patients (18.3%) developed a primary nosocomial BSI within 30 days after the CDI episode (CDI/BSI$^+$ group), and these patients were compared to 321 patients with CDI but no evidence of primary BSI during hospitalization (CDI/BSI$^-$ group). There were no significant differences between the two study groups in terms of ward of hospitalization (medical wards, 63% CDI/BSI$^+$ versus 62% CDI/BSI$^-$ [$P = 0.8$]; ICU, 19% CDI/BSI$^+$ versus 17% CDI/BSI$^-$ [$P = 0.7$]; surgical wards, 18% CDI/BSI$^+$ versus 21% CDI/BSI$^-$ [$P = 0.3$]).

Table 1 reports the pathogens causing BSI in patients of the CDI/BSI$^+$ group. The most common etiology was *Candida* species (47.3%), followed by enterobacteria (19.4%), mixed infections (19.4%), and enterococci (13.9%). Among patients with monomicrobial or polymicrobial bacterial BSI, an MDR phenotype was detected in 26 out of 38 cases (68.4%), with 11 cases of extended-spectrum beta-lactamases (ESBL) producing *Enterobacteriaceae*, 8 cases of carbapenemase-producing *Klebsiella pneumoniae*, and 7 cases of vancomycin-resistant enterococci (VRE).

Demographics and clinical features of CDI patients with or without nosocomial BSI are summarized in Table 2. No differences were detected in terms of age, sex, and comorbidities (also calculated as a Charlson score) between the two study groups. Compared to the CDI/BSI$^-$ group, patients included in the CDI/BSI$^+$ group were more frequently affected by a severe CDI (100%

TABLE 3 Initial antibiotic regimen and outcomes for patients with CDI/BSI compared to those of controls

Variable	Value(s) by infection status		*P* value[a]
	CDI/BSI$^+$ ($n = 72$)	CDI/BSI$^-$ ($n = 321$)	
Oral vancomycin (no. [%])	61 (84.7)	262 (81.6)	0.7
Metronidazole (no. [%])	11 (15.3)	59 (18.4)	0.4
Escalation to oral vancomycin + metronidazole (no. [%])	25 (34.7)	95 (29.6)	0.1
Oral vancomycin, >500 mg/day (no. [%])	51 (70.8)	100 (31.1)	**<0.001**
Transfer to ICU (no. [%])	14 (19.5)	29 (9)	**0.002**
Days of ICU stay (no.)	16.9 ± 4.4	9.1 ± 4.9	**<0.001**
Days of hospital stay (no.)	62.2 ± 21.9	29.3 ± 13.2	**<0.001**
Time at risk for CDI (days)	9.5 ± 1.4	11.1 ± 2.3	0.2
Time at risk for CDI recurrence (days)	20.4 ± 3.3	35.1 ± 6.2	**<0.001**
Severe sepsis or septic shock (no. [%])	61 (84.7)	50 (15.7)	**<0.001**
30-Day mortality from CDI diagnosis (no. [%])	28 (38.9)	42 (13.1)	**0.001**
Mortality attributable to BSI (no. [%])	41 (56.9)		
All causes of in-hospital mortality (no. [%])	55 (76.3)	70 (21.8)	**<0.001**

[a] Values in boldface are significant.

versus 46.7%; $P < 0.001$) and had a higher rate of *C. difficile* recurrence (83.3% versus 29.6%; $P < 0.001$), a higher frequency of ≥1 recurrences (33.3% versus 7.1%; $P < 0.001$), and a higher median SOFA score (3.6 versus 1.7; $P < 0.001$). Compared to patients of the CDI/BSI$^-$ group, those included in the CDI/BSI$^+$ group had a higher frequency of ribotype 027 infection (84.7% versus 33.9%; $P < 0.001$); patients with CDI due to ribotype 027 had a high likelihood of developing BSI during the first 2 weeks (94% versus 21%) from the initial CDI diagnosis.

Table 3 describes antibiotic regimens and outcomes for patients with CDI. All patients initially were treated with vancomycin or metronidazole monotherapy; during hospital stay an escalation therapy, including oral vancomycin plus metronidazole, was recorded in 34.7% of patients of the CDI/BSI$^+$ group and in 29.6% of those of the CDI/BSI$^-$ group. Overall, a dosage of oral vancomycin of >500 mg/day was used in 51 (70.8%) patients of the CDI/BSI$^+$ group and 100 (31.1%) patients of the control group ($P < 0.001$). Among patients receiving increased vancomycin dosages, the following regimens were adopted: 250 mg three times a day in 87 cases and 250 mg four times a day in 64 patients. Patients of the CDI/BSI$^+$ group also had longer ICU stays (16.9 versus 9.1 days; $P < 0.001$) and hospital lengths of stay (62.2 versus 29.3 days; $P < 0.001$). Of interest, patients of the CDI/BSI$^+$ group showed a shorter time at risk for CDI recurrence (20.4 versus 35.1 days; $P < 0.001$). The median time at risk for primary BSI in the CDI/BSI$^+$ group was 14.8 ± 2.9 days.

Overall, 30-day mortality was 17.8% among all patients with CDI; 30-day mortality from CDI diagnosis was higher in patients of the CDI/BSI$^+$ group than for controls (38.9 versus 13.1%; $P < 0.001$), and in 41 (56.9%) patients of the CDI/BSI$^+$ group, mortality was attributable to primary BSI. BSI-attributable mortality was 51.1% in patients with bacterial infection, 67.6% in those with *Candida* infection, and 50% in those with mixed infection. Thirty-day mortality in patients with CDI due to ribotype 027 was 71% in patients of the CDI/BSI$^+$ group and 26% in patients of the CDI/BSI$^-$ group.

Finally, Table 4 shows results of the multivariate analysis, and ribotype 027 infection status, CDI recurrence, severity of CDI infection, and oral vancomycin (at >500 mg/day) were risk factors independently associated with the development of BSI after CDI.

DISCUSSION

Our study is the first to describe an association between CDI and nosocomial BSI. The novel message is that a significant percentage of patients with CDI may develop a primary BSI, mostly caused by *Candida* or enteric bacteria, and that mortality associated with this complication is very high, exceeding 50%.

From a clinical standpoint, our findings reveal that clinicians should be very diligent in diagnosing and treating a BSI during the first 2 to 4 weeks after CDI diagnosis, since this complication is associated with an excess of mortality. In a multicenter cohort study, CDI mortality was 13% after 30 days and 37% after 1 year (27). We observed a slightly higher mortality in our population (17.8%), probably because we included elderly patients with multiple comorbidities and with a high frequency of CDI recurrence. This finding may be explained by the fact that the hospitals involved in this study provide assistance to patients following transfer from various nursing homes, long-term-care facilities, and community hospitals of our region, so probably we analyzed a setting predisposed to severely ill and frail patients with multiple risk factors for infection and high frequencies of previous antibiotic therapy.

We have recently demonstrated that patients with CDI may suffer from subsequent *Candida* BSI (10). The alterations to the intestinal mucosa and resident flora occurring in patients with CDI may predispose them to the translocation of pathogens from the intestinal lumen to the blood, particularly in patients with severe CDI and/or CDI recurrences, as a consequence of two main factors: (i) the severe mucosal damage associated with ribotype 027 *C. difficile* strains and (ii) the impairment of the normal intestinal microbiota due to prolonged vancomycin therapy. As a mat-

TABLE 4 Multivariate analysis of factors associated with primary BSI during CDI

Variable	*P* value	OR	95% CI
Ribotype 027	<0.001	6.5	3.99–9.12
CDI recurrence	<0.001	5.5	3.11–11.23
Severe CDI infection	<0.001	8.3	4.76–14.12
Oral vancomycin, >500 mg/day	<0.001	3.1	1.57–4.67

ter of fact, the receipt of high oral vancomycin dosages and ribotype 027 infection were independent risk factors for developing a BSI.

Of importance, all cases of primary BSI were caused by enteric pathogens like *Candida*, *Enterobacteriaceae*, or enterococci. Since patients with other documented foci of infection were excluded, microbial translocation from the gut was the likely source of infection in all cases. Three conditions usually are necessary for the hematogenous spread of microorganism residents in the gut: alterations of the normal integrity of the mucosal epithelium, impairment of mucosal immunity (particularly neutrophils, which play a crucial role in clearing gastrointestinal candidiasis), and colonization of gastrointestinal mucosa. Among patients with severe CDI, all of the above-described conditions coexist frequently. Mucosal damage is sustained by an intense host inflammatory response, particularly in those with the 027 ribotype (28, 29), and this condition frequently persists despite the administration of appropriate antibiotic therapy (30). Moreover, toxin production exerts mucosal immunity impairment by the modification of neutrophil morphology and function (31). Moreover, a significant number of patients of the CDI/BSI$^+$ group received immunosuppressive therapies, especially steroids, and this factor can be involved in an increased susceptibility to invasive infections.

CDI may promote colonization by *Candida* and other microorganisms: Raponi et al. (32) showed that CDI is significantly associated with *Candida* colonization. Furthermore, Nerandzic and coworkers found high rates of stool colonization by *Candida* species and/or vancomycin-resistant enterococci after oral vancomycin therapy (12). Of interest, the majority of our patients with bacterial BSI had an MDR etiology (mostly ESBLs and carbapenemase-producing *K. pneumoniae*), highlighting the role of the intestinal tract as a reservoir of MDR organisms in patients with multiple health care contacts (33). Along these lines, our data confirm the recent experience of Amit et al. who found CDI was a predisposing factor for Gram-negative BSI (34).

Another crucial point of our study is the association between BSI and ribotype 027 infection. During recent years an increasing incidence of ribotype 027 has been reported in our geographic area (35), and there is epidemiological evidence of a recent spread of this organism in our hospital (36). In our study, CDI due to ribotype 027 is associated with an increased risk of BSI during the first 30 days after diagnosis and increased 30-day mortality. This finding supports the hypothesis that the hypervirulent ribotype 027 strains cause major damage to the integrity of intestinal mucosa, favoring the translocation of microbes to the blood. Furthermore, oral vancomycin, especially if higher dosages are used, also may cause delayed intestinal tissue injury that may act as an additional driver for microbial translocation (37). Our data suggest avoiding the use of increased oral vancomycin dosages and confirm previous observations (38).

There are four important limitations to our observations: first, the retrospective nature of the study does not allow definitive conclusions, and future large trials will be necessary to confirm our data; second, the possible microbial colonization preceding CDI was not assessed in the population; third, it is possible that the association between severe CDI and nosocomial BSI can be detected in an old and frail patient population, as depicted by the present study, but our findings cannot be generalized to all patients with CDI; fourth, the significantly higher use of immunosuppressive therapy in the CDI/BSI$^+$ group may make these patients susceptible to invasive infections. However, despite these limitations, our analyses provide a strong rationale for a possible link between severe CDI and BSI.

In conclusion, it is possible to hypothesize an increased risk of BSI in patients with severe or relapsing CDI. The evidence from our study highlights the leading role of ribotype 027 strains and higher oral vancomycin dosages in promoting nosocomial BSI. Our findings confirm the need for further and more comprehensive approaches to treating patients with CDI.

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REFERENCES

1. **Bartlett JG, Gerding DN.** 2008. Clinical recognition and diagnosis of *Clostridium difficile* infection. Clin Infect Dis **46:**S12–S18. http://dx.doi.org/10.1086/521863.
2. **Crobach MJ, Dekkers OM, Wilcox MH, Kuijper EJ.** 2009. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): data review and recommendations for diagnosing Clostridium difficile-infection (CDI). Clin Microbiol Infect **15:**1053–1066. http://dx.doi.org/10.1111/j.1469-0691.2009.03098.x.
3. **Bauer MP, Kuijper EJ, van Dissel JT.** 2009. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): treatment guidance for *Clostridium difficile* infection (CDI). Clin Microbiol Infect **15:**1067–1079. http://dx.doi.org/10.1111/j.1469-0691.2009.03099.x.
4. **Wilcox MH, Mooney L, Bendall R, Settle CD, Fawley WN.** 2008. A case-control study of community-associated *Clostridium difficile* infection. J Antimicrob Chemother **62:**388–396. http://dx.doi.org/10.1093/jac/dkn163.
5. **Kelly CP, LaMont JT.** 2008. Clostridium difficile: more difficult than ever. N Engl J Med **359:**1932–1940. http://dx.doi.org/10.1056/NEJMra0707500.
6. **Kuijper EJ, Coignard B, Tüll P, ESCMID Study Group for Clostridium difficile, EU Member States, European Centre for Disease Prevention and Control.** 2006. Emergence of *Clostridium difficile*-associated disease in North America and Europe. Clin Microbiol Infect **12:**2–18.
7. **Andes DR, Safdar N, Baddley JW, Playford G, Reboli AC, Rex JH, Sobel JD, Pappas PG, Kullberg BJ, Mycoses Study Group.** 2012. Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis: a patient-level quantitative review of randomized trials. Clin Infect Dis **54:**1110–1122. http://dx.doi.org/10.1093/cid/cis021.
8. **Guastalegname M, Russo A, Falcone M, Giuliano S, Venditti M.** 2013. Candidemia subsequent to severe infection due to *Clostridium difficile*: is there a link? Clin Infect Dis **57:**772–774. http://dx.doi.org/1093/cid/cit362.
9. **Guastalegname M, Grieco S, Giuliano S, Falcone M, Caccese R, Carfagna P, D'ambrosio M, Taliani G, Venditti M.** 2014. A cluster of fulminant *Clostridium difficile* colitis in an intensive care unit in Italy. Infection **42:**585–589. http://dx.doi.org/10.1007/s15010-014-0597-1.
10. **Russo A, Falcone M, Fantoni M, Murri R, Masucci L, Carfagna P, Ghezzi MC, Posteraro B, Sanguinetti M, Venditti M.** 2015. Risk factors and clinical outcomes of candidaemia in patients treated for *Clostridium difficile* infection. Clin Microbiol Infect **21:**493. http://dx.doi.org/10.1016/j.cmi.2014.12.024.
11. **Giuliano S, Guastalegname M, Jenco M, Morelli A, Falcone M, Venditti M.** 2014. Severe community onset healthcare-associated *Clostridium difficile* infection complicated by carbapenemase producing *Klebsiella pneumoniae* bloodstream infection. BMC Infect Dis **14:**475. http://dx.doi.org/10.1186/1471-2334-14-475.
12. **Nerandzic MM, Mullane K, Miller MA, Babakhani F, Donskey CJ.** 2012. Reduced acquisition and overgrowth of vancomycin-resistant enterococci and *Candida* species in patients treated with fidaxomicin versus vancomycin for *Clostridium difficile* infection. Clin Infect Dis **55:**S121–S126. http://dx.doi.org/10.1093/cid/cis440.
13. **Manian FA, Bryant A.** 2013. Does *Candida* species overgrowth protect against *Clostridium difficile* infection? Clin Infect Dis **56:**464–465. http://dx.doi.org/10.1093/cid/cis854.
14. **Shankar V, Hamilton MJ, Khoruts A, Kilburn A, Unno T, Paliy O, Sadowsky MJ.** 2014. Species and genus level resolution analysis of gut

microbiota in *Clostridium difficile* patients following fecal microbiota transplantation. Microbiome **2:**13. http://dx.doi.org/10.1186/2049-2618-2-13.

15. **Falcone M, Russo A, Iraci F, Carfagna P, Goldoni P, Vullo V, Venditti M.** 2015. Abstr 55th Intersci Conf Antimicrob Agents Chemother, oral communication no. 1089.
16. **Cohen SH, Gerding DN, Johnson S, Kelly CP, Loo VG, McDonald LC, Pepin J, Wilcox MH, Society for Healthcare Epidemiology of America, Infectious Diseases Society of America.** 2010. Clinical practice guidelines for *Clostridium difficile* infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). Infect Control Hosp Epidemiol **31:**431. http://dx.doi.org/10.1086/651706.
17. **Horan TC, Andrus M, Dudeck MA.** 2008. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control **36:**309–332. http://dx.doi.org/10.1016/j.ajic.2008.03.002.
18. **Pappas PG, Kauffman CA, Andes D, Benjamin DK, Jr, Calandra TF, Edwards JE, Jr, Filler SG, Fisher JF, Kullberg BJ, Ostrosky-Zeichner L, Reboli AC, Rex JH, Walsh TJ, Sobel JD, Infectious Diseases Society of America.** 2009. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis **48:**503–535. http://dx.doi.org/10.1086/596757.
19. **Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb S, Beale RJ, Vincent JL, Moreno R, Surviving Sepsis Campaign Guidelines Committee including The Pediatric Subgroup.** 2013. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med **39:**165–228. http://dx.doi.org/10.1007/s00134-012-2769-8.
20. **O'Grady NP, Alexander M, Dellinger EP, Gerberding JL, Heard SO, Maki DG, Masur H, McCormick RD, Mermel LA, Pearson ML, Raad II, Randolph A, Weinstein RA, Healthcare Infection Control Practices Advisory Committee.** 2002. Guidelines for the prevention of intravascular catheter-related infections. Am J Infect Control **30:**476–489. http://dx.doi.org/10.1067/mic.2002.129427.
21. **León C, Alvarez-Lerma F, Ruiz-Santana S, León MA, Nolla J, Jordá R, Saavedra P, Palomar M, EPCAN Study Group.** 2009. Fungal colonization and/or infection in non-neutropenic critically ill patients: results of the EPCAN observational study. Eur J Clin Microbiol Infect Dis **28:**233–242. http://dx.doi.org/10.1007/s10096-008-0618-z.
22. **Falcone M, Russo A, Giannella M, Cangemi R, Scarpellini MG, Bertazzoni G, Alarcón JM, Taliani G, Palange P, Farcomeni A, Vestri A, Bouza E, Violi F, Venditti M.** 2015. Individualizing risk of multidrug-resistant pathogens in community-onset pneumonia. PLoS One **10:**e0119528. http://dx.doi.org/10.1371/journal.pone.0119528.
23. **Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL.** 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect **18:**268–281. http://dx.doi.org/10.1111/j.1469-0691.2011.03570.x.
24. **Loo VG, Poirier L, Miller MA, Oughton M, Libman MD, Michaud S, Bourgault AM, Nguyen T, Frenette C, Kelly M, Vibien A, Brassard P, Fenn S, Dewar K, Hudson TJ, Horn R, René P, Monczak Y, Dascal A.** 2005. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. N Engl J Med **353:**2442–2449. http://dx.doi.org/10.1056/NEJMoa051639.
25. **Pittet D, Tarara D, Wenzel RP.** 1994. Nosocomial bloodstream infection in critically ill patients. Excess length of stay, extra costs, and attributable mortality. JAMA **271:**1598–1601.
26. **Spigaglia P, Mastrantonio P.** 2002. Molecular analysis of the pathogenicity locus and polymorphism in the putative negative regulator of toxin production (TcdC) among *Clostridium difficile* clinical isolates. J Clin Microbiol **40:**3470–3475. http://dx.doi.org/10.1128/JCM.40.9.3470-3475.2002.
27. **Hensgens MP, Goorhuis A, Dekkers OM, van Benthem BH, Kuijper EJ.** 2013. All-cause and disease-specific mortality in hospitalized patients with *Clostridium difficile* infection: a multicenter cohort study. Clin Infect Dis **56:**1108–1116. http://dx.doi.org/10.1093/cid/cis1209.
28. **Madan R, Petri WA, Jr.** 2012. Immune responses to *Clostridium difficile* infection. Trends Mol Med **18:**658–666. http://dx.doi.org/10.1016/j.molmed.2012.09.005.
29. **Chumbler NM, Farrow MA, Lapierre LA, Franklin JL, Haslam DB, Goldenring JR, Lacy DB.** 2012. *Clostridium difficile* toxin B causes epithelial cell necrosis through an autoprocessing-independent mechanism. PLoS Pathog **8:**e1003072. http://dx.doi.org/10.1371/journal.ppat.1003072.
30. **El Feghaly RE, Stauber JL, Deych E, Gonzalez C, Tarr PI, Haslam DB.** 2013. Markers of intestinal inflammation, not bacterial burden, correlate with clinical outcomes in Clostridium difficile infection. Clin Infect Dis **56:**1713–1721. http://dx.doi.org/10.1093/cid/cit147.
31. **Brito GA, Sullivan GW, Ciesla WP, Jr, Carper HT, Mandell GL, Guerrant RL.** 2002. *Clostridium difficile* toxin A alters in vitro-adherent neutrophil morphology and function. J Infect Dis **185:**1297–1306.
32. **Raponi G, Visconti V, Brunetti G, Ghezzi MC.** 2014. Clostridium difficile infection and candida colonization of the gut: is there a correlation? Clin Infect Dis **59**(11)**:**1648–1649. http://dx.doi.org/10.1093/cid/ciu637.
33. **De Rosa FG, Corcione S, Pagani N, Di Perri G.** 2015. From ESKAPE to ESCAPE, from KPC to CCC. Clin Infect Dis **60:**1289–1290. http://dx.doi.org/10.1093/cid/ciu1170.
34. **Amit S, Mishali H, Kotlovsky T, Schwaber MJ, Carmeli Y.** 2015. Bloodstream infections among carriers of carbapenem-resistant *Klebsiella pneumoniae*: etiology, incidence and predictors. Clin Microbiol Infect **21:**30–34. http://dx.doi.org/10.1016/j.cmi.2014.08.001.
35. **Di Bella S, Paglia MG, Johnson E, Petrosillo N.** 2012. *Clostridium difficile* 027 infection in central Italy. BMC Infect Dis **12:**370. http://dx.doi.org/10.1186/1471-2334-12-370.
36. **Orsi GB, Conti C, Mancini C, Giordano A.** 2014. Clostridium difficile 027 increasing detection in a teaching hospital in Rome, Italy. Infection **42:**941–942. http://dx.doi.org/10.1007/s15010-014-0652-y.
37. **Warren CA, van Opstal EJ, Riggins MS, Li Y, Moore JH, Kolling GL, Guerrant RL, Hoffman PS.** 2013. Vancomycin treatment's association with delayed intestinal tissue injury, clostridial overgrowth, and recurrence of *Clostridium difficile* infection in mice. Antimicrob Agents Chemother **57:**689–696. http://dx.doi.org/10.1128/AAC.00877-12.
38. **Lam SW, Bass SN, Neuner EA, Bauer SR.** 2013. Effect of vancomycin dose on treatment outcomes in severe Clostridium difficile infection. Int J Antimicrob Agents **42:**553–558. http://dx.doi.org/10.1016/j.ijantimicag.2013.08.013.