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# Clostridium Difficile Infection in Hospitalized Patients at University Clinical Center Tuzla, Bosnia and Herzegovina: a 4 Year Experience

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## ABSTRACT

**Introduction:** Clostridium difficile (*C. difficile*) is currently the leading cause of healthcare-associated diarrhea, but almost nothing is known about the extent of *C. difficile* infection (CDI) in Bosnia and Herzegovina. **Goal:** We aimed to retrospectively analyze CDI in hospitalized patients at University Clinical Center (UCC) Tuzla, Bosnia and Herzegovina from January 2009 through June 2012. **Methods:** We analyzed all patients (except children ages 0-2), diagnosed with CDI based on anamnestic and epidemiological, clinical picture and microbiological tests (proof of toxins in the stool by enzyme-linked immunosorbent assay). **Results:** From a total of 989 patients tested for *C. difficile* toxin (60.2 per 10,000 inpatient days) 347 (35.08%) were positives. The mean incidence rate of CDI was 2.23 per 10,000 inpatient days (range 1.32-2.87). Annual rates of hospitalization were 15.68 per 10,000 admissions (range 8.99-20.35). Most patients had a previously identified risk profile of old age, comorbidity and recent use of antibiotics. 41/276 (14.86%) patients had died, and 11/41 (26.82%) were CDI-associated deaths. Complicated CDI were registered in 53/276 (19.21%) patients, and recurrent infections in 65/276 (23.55%). **Conclusion:** Our data suggest that CDI is largely present in our setting which represents a serious problem and points to the importance of international surveillance, detection and control of CDI.

**Key words:** Clostridium difficile infection, healthcare-associated, incidence rate, diarrhea.

## 1. INTRODUCTION

*Clostridium difficile* (*C. difficile*) is currently the leading cause of healthcare-associated diarrhea with potentially fatal outcomes. The incidence and severity of *C. difficile* infection (CDI) throughout the world has increased in the last 20 years due to the emergence of hypervirulent strains, increased use and misuse of antibiotics, as well as increased susceptibility in a population at-risk, and other risk factors. Various studies in Canada, the United States (U.S.) and Europe recorded an increase of 2 to 4-fold in CDI incidence (1, 2). As the incidence of CDI increased, CDI mortality and colectomy rates increased as well (3). Recent data from the European studies show a mean incidence rate of healthcare-associated CDI of 4.1 per 10,000 inpatient days, but ranged from 0.0 to 36.3 (4).

There is abundance of data on CDI prevalence in Europe, however little is known about the prevalence of CDI in Eastern Europe, and almost nothing about prevalence in Bosnia and Herzegovina. Therefore we aimed to retrospectively analyze

epidemiological, clinical and microbiological characteristics of patients with CDI hospitalized in clinics at University Clinical Center (UCC) Tuzla, Bosnia and Herzegovina from January 2009 through June 2012.

## 2. METHODS

### Study design and patients

We retrospectively collected and analyzed epidemiological, clinical and microbiological data for 276 patients, who were during hospitalization in clinics at UCC Tuzla, in the period from 1.1.2009 to 30.6.2012, diagnosed and treated for CDI. The diagnosis of CDI was determined based on anamnestic data, epidemiological data, clinical picture (basic clinical symptoms and signs), microbiological tests (enzyme-linked immunosorbent assay (ELISA) for detection of *C. difficile* toxin A and B; Serazym *Clostridium difficile* Toxin A+B, Seramun Diagnostica GmbH, Heidesee, Germany), and according to the definition of guidelines for CDI treatment by the European Association

	Number of toxin-positive cases/ number of patients tested	Number of patient tested per 10,000 inpatient days	Number of toxin-positive cases whom questionnaires were completed	Weighted mean of healthcare-associated <i>C. difficile</i> infection incidence rate per clinic (minimum to maximum range) per 10,000 inpatient days per 10,000 admissions
Clinic of General and Abdominal Surgery	30/74 (40.54%)	44.18	45/276 (16.30%)	4.14 (2.61-9.97) 26.87 (12.71-63.21)
Clinic of Pulmonary Diseases	48/89 (53.92%)	144.88	32/276 (11.59%)	2.84 (0.93-6.30) 52.09 (6.36-114.55)
Clinic of Orthopedics and Traumatology	58/98 (59.18%)	127.14	53/276 (19.20%)	8.41 (3.25-13.81) 68.76 (23.84-111.36)
Internal Medicine Hospital	86/299 (28.76%)	127.43	74/276 (26.81%)	3.36 (1.90-4.90) 31.54 (17.37-47.68)
Children's Hospital	2/20 (10.60%)	17.53	1/276 (0.36%)	0.16 (0.00-0.59) 0.88 (0.00-3.26)
Clinic of Otorhinolaryngology	3/7 (42.85%)	15.02	6/276 (1.08%)	1.76 (0.00-4.71) 8.58 (0.00-7.83)
Clinic of Physical Medicine and Rehabilitation	15/35 (42.85%)	148.05	12/276 (4.34%)	2.26 (0.96-3.30) 50.76 (6.36-77.12)
Clinic of Oncology, Hematology and Radiotherapy	6/24 (25.00%)	55.66	3/276 (1.08%)	0.57 (0.00-1.22) 6.96 (0.00-14.59)
Clinic of Cardiovascular Diseases	7/27 (25.92%)	38.45	13/276 (4.71%)	4.08 (2.00-8.93) 18.51 (4.77 - 40.00)
Clinic of Plastic and Maxillofacial Surgery	1/1 (100.00%)	2.59	1/276 (0.36%)	0.69 (0.00-2.04) 2.59 (0.00-7.38)
Clinic of Neurology	2/7 (28.57%)	12.42	2/276 (0.72%)	0.34 (0.00-2.49) 3.55 (0.00-24.63)
Clinic of Neurosurgery	4/10 (40.00%)	47.26	7/276 (2.53%)	4.24 (3.63-5.33) 33.08 (4.77-32.68)
Clinic of Anesthesiology and Reanimation	23/54 (42.59%)	191.49	6/276 (2.17%)	2.81 (0.00-7.47) 21.28 (0.00-11.48)
Infectious Diseases Hospital	61/234 (26.06%)	434.22	1/276 (0.36%)	0.19 (0.00-0.65) 18.6 (0.00-1.59)
Clinic of Gynecology and Obstetrics	0/4 (0.00%)	0.95	2/276 (0.72%)	0.16 (0.00-0.59) 0.47 (0.00-1.71)
Outpatients			20/276 (7.25%)	
Total (Clinical Center Tuzla)	347/989 (35.08%)	60.20	276/276(100%)	2.23 (1.32 - 2.87) 15.68 (8.99-20.35)

Table 1. Summary of Clostridium difficile infection in clinics University Clinical Center Tuzla 2009.-2012.

of Clinical Microbiology and Infectious Diseases (ESCMID) (5). We also collected data from the Institute of Microbiology UCC Tuzla; the total number of toxin-positive cases and the number of patients tested, as well as the data from the Office of Planning and Analysis UCC Tuzla; the number of hospitalized patients and the number of inpatient days.

#### Statistical analysis

All statistical analyses were performed using SPSS 15.0 (SPSS, Chicago, Illinois, USA). Descriptive statistical parameters have been used for the determination of baseline characteristics. We calculated intra hospital prevalence based on 10,000 inpatient days. A statistical level of 95% ( $P < 0.05$ ) was considered significant for all performed tests.

### 3. RESULTS

From January 2009 through June 2012, 347 patients were admitted and treated for CDI at UCC Tuzla. The total number of toxin-positive cases amounted to 347 out of 989 patients tested with suspected CDI (35.08%) (Table 1). The number of patients tested per 10,000 inpatient days was 60.2 (Table 1). 276/347 (79.53%) patients were included in this study: who had toxin-positive-stool samples and available clinical and epidemiological data. Most cases were healthcare-associated infections 256/276 (92.75%), while 20/276 (7.25%) were the outpatient cases. Healthcare-associated infections were registered in 15/19 (78.94%) clinics at UCC Tuzla, while in the remaining four clinics (21.06%) there were no patients with CDI. The incidence rate of CDI varied across clinics, weighted mean 2.23 per 10,000 inpatient days (range 1.32-2.87) (Table 1).

Three leading clinics with the highest incidence rate of CDI per 10,000 inpatient days were: the Clinic of Orthopedics and Traumatology, weighted mean 8.41 (range 3.25-13.81), the

Clinic of General and Abdominal Surgery, weighted mean 4.14 (range 2.61-9.97) and the Clinic of Cardiovascular Diseases, weighted mean 4.08 (range 2.00- 8.93).

Our research has shown that the annual rates of hospitalization with CDI during were 15.68 per 10,000 admissions (range 8.99-20.35). Three leading clinics with registered and hospitalized patients with CDI were: the Clinic of Orthopedics and Traumatology, weighted mean 68.76 per 10,000 admissions (range 23.84-111.36), then the Clinic of Pulmonary Diseases and Tuberculosis, 52.09 (range 6.36- 114.55), and the Clinic of Physical Medicine and Rehabilitation, 50.76 (range 6.36-77.12). The largest number of hospitalized patients after evidence of healthcare-associated CDI infection was transferred to the Infectious Diseases Hospital 142/276 (51.44%), for further treatment.

Most patients fit the previously determined risk profile: 174 (63.04%) were older than 65 years, 148 (53.62%) had severe comorbidity, 139 (49.64%) had a surgical procedure in the previous 3 months, and most of them, 256 (92.5%), received antibiotics 3 months prior to infection; usually cephalosporines (71.02%), quinolones (32.97%) or different combinations of antibiotics (Table 2).

250/276 (90.57%) CDI patients were receiving antibiotics within 1 month before the onset of symptoms. 5/276 (1.81%) patients had recurrent CDI at inclusion. Before hospital admission, 20/276 (7.25%) patients were infected with *C. difficile* in the outpatient setting. In 222/276 (80.43%) patients, CDI was clinically manifested as a short diarrheal disease before taking stool samples, less than 1 week. Furthermore, 117/276 (42.93%) patients had fever, diarrhea mixed with blood 19/276 (6.88%), ileus 7/276 (2.54%) in the week before taking stool samples (Table 2).

n/ N (%)	
Epidemiological characteristics	
Male	141/276 (51.09%)
Age ≥65 years	174/276 (63.04%)
Epidemiological association	
Healthcare-associated	256/276 (92.75%)
Community-associated	20/276 (7.25%)
Explicit request to test for infection	249/276 (90.22%)
Use of an antibiotic not directed at <i>C. difficile</i> infection during previous 3 months	256/276 (92.5%)
Any antibiotic not directed at <i>C. difficile</i> infection	
Natural penicillin	4/276 (1.45%)
Penicillinase - resistant penicillin	1/276 (0.36%)
Aminopenicillin	14/276 (5.07%)
Aminopenicillin-β-lactamase inhibitor combination	18/276 (6.52%)
Antipseudomonal penicillin-β-lactamase inhibitor combination	1/276 (0.36%)
First-generation cephalosporin	93/276 (33.70%)
Third-generation cephalosporin	86/276 (31.16%)
Ceftazidime	3/276 (1.09%)
Any cephalosporin	196/276 (71.02%)
Carbapenem	21/276 (7.61%)
Aminoglycoside	28/276 (10.14%)
Old quinolone	87/276 (31.52%)
New quinolone	4/276 (1.45%)
Intravenous glycopeptide	11/276 (3.99%)
Lincosamide	2/276 (0.72%)
Macrolide	3/276 (1.09%)
Co-trimoxazole	22/276 (7.97%)
Use of any antibiotic not directed at <i>C. difficile</i> infection in previous month	250/276 (90.57%)
Comorbidity	
Severe comorbidity (APACHE II CHP > 0)	148/276 (53.62%)
Liver cirrhosis (APACHE II)	9/276 (3.26%)
Heart disease (APACHE II)	87/276 (31.52%)
Pulmonary disease (APACHE II)	62/276 (22.46%)
Chronic dialysis (APACHE II)	21/276 (7.61%)
Immunocompromised status (APACHE II)	119/276 (43.12%)
Treatment for inflammatory bowel disease	9/276 (3.26%)
Episodes of infection in previous 8 weeks	5/276 (1.81%)
Surgical treatment during previous 3 months	137/276 (49.64%)
Disease characteristics	
Outpatient	20/276 (7.25%)
Duration of diarrhea	
<1 week	222/276 (80.43%)
1 to 3 weeks	42/276 (15.22%)
>3 weeks	12/276 (4.35%)
Diarrhea mixed with blood at any moment in previous week	19/276 (6.88%)
Fever (temperature >38.5°C)	117/276 (42.39%)
Ileus at any moment in previous week	7/276 (2.54%)
Last leucocyte count in previous week ≥15 × 10 <sup>9</sup> /L	85/276 (30.79%)
Serum creatinine rise >50% compared to baseline before onset of symptoms	73/276 (26.44%)
Rectosigmoidoscopy or colonoscopy	10/276 (3.62%)
Pseudomembranes	8/10 (80%)
Ulceration	9/10 (90%)
Imaging	
Colonic wall thickening on CT	13/24 (54.1%)

Pericolonic fat stranding on CT	6/24 (25.00%)
Bowel distension on plain abdominal radiograph or CT	15/40 (37.50%)
Microbiological characteristics	
ELISA test for Clostridium difficile toxin A/B	276/276 (100%)

Table 2. Characteristics of patients with Clostridium difficile infection

In 73/276 (26.44%) patients, the most recent laboratory results of serum creatinine before taking stool samples were increased by more than 50% compared with the last values before the onset of CDI symptoms. Leukocyte count during the week prior to sampling was  $\geq 15 \times 10^9/L$  in 85/276 (30.79%) patients. In moderate and severe cases of CDI, the disease was manifested with colitis, a large number of stools (at times mucous and bloody), dehydration, sometimes severe abdominal pain and meteorism. 10/276 (3.62%) patients underwent rectosigmoidoscopy or colonoscopy. Thus, in 8/10 (80%) patients we detected pseudo membranes and ulcerations in 9/10 (90%). Also, in the first radiography or computed tomography (CT) image bowel distension was observed in 15/40 (37.50%) patients.

In the course of this analysis, of the total number of CDI patients, hospitalized and confirmed by ELISA for *C. difficile* toxins, treatment and outcome data were collected for 276/347 (79.5%). From 41/276 (14.86%) patients who died, in 11/41 (26.82%) death was CDI-associated (Table 3).

All 11 patients whose death was due primarily to *C. difficile* infection were between 70 to 91 years old, and their infection was healthcare-associated. They all had severe comorbidity, 1 to 3 different diseases, and 6 of them besides comorbidity had previous surgical interventions within 3 months before the onset of symptoms.

Colectomy was performed in 12/276 (4.35%) patients. Complicated CDI were registered in 53/276 (19.21%), while recurrent infections in 65/276 (23.55%) patients. Treatment of CDI patients consisted of discontinuation of antimicrobial therapy, changing antimicrobial agent, diet and rehydration, and other supportive therapies. The initial episode of CDI in our patients was treated with oral metronidazole in 129/276 (50.36%). Since

n/ N (%)	
Initial episode treated with	
Oral metronidazole	129/276 (46.74%)
Intravenous metronidazole	10/276 (3.63%)
Oral vancomycin	139/276 (50.36%)
Intravenous vancomycin	2/276 (0.72%)
ICU admissions	157/276 (56.87%)
CDI contributive	36/276 (13.04%)
CDI primary cause	111/276 (40.21%)
Colectomy for CDI	12/276 (4.35%)
Death	41/276 (14.86%)
CDI contributive	23/276 (8.33%)
CDI primary cause	10/276 (3.62%)
CDI no relationship	8/276 (2.90%)
Complicated CDI	53/276 (19.21%)
Recurrent CDI	65/276 (23.55%)
Both complicated and recurrent CDI	118/276 (42.76%)

Table 3. Treatment and outcome characteristics of patients with Clostridium difficile infection

CDI infections were mainly healthcare-associated, occurred in different, most clinics at UCC Tuzla and represented a serious problem in the past, a large percentage of patients with CDI were isolated and treated in the intensive care unit, a total of 157/276 (56.8%).

#### 4. DISCUSSION

Reports from many European countries (Finland, Denmark, and Germany) (6-8), and elsewhere (2, 9, 10), record the increment in CDI incidence. Some authors report reduction in CDI incidence and CDI-associated mortality due to improved surveillance, reporting and prevention (11). According to reports from the U.S., the rate of pediatric CDI hospitalizations increased from 7.2 to 12.80 from 1997 through 2006; the lowest rate was for children under age 1 [9]. Approximately 11 – 28 % of CDI patients acquired infection in the community, which seems consistent in different countries (12, 13). It is known that the main cause of CDI is exposure to antibiotics or chemotherapeutics (antineoplastic agents), then high age, comorbidity and hospital stay. Just recently additional risk factors for acquiring CDI have been reported: inflammatory bowel disease – ulcerative colitis and Crohn's disease, pharmacological blockade of gastric acid secretion caused primarily by proton pump inhibitors, organ transplantation, previous gastrointestinal surgery and others (1, 14, 15).

*C. difficile* causes pathogenic diseases of the gastrointestinal tract by secreting 2 exotoxins, enterotoxin A and cytotoxin B, which cause diarrhea and colitis. For a long time, it has been thought that *C. difficile* produces both toxins, but the latest research suggests the existence of strains that produce only toxin B. It is estimated that the changes in the flora of the colon and intestinal motility dysfunction represent a suitable basis for the development of infection (16, 17).

*C. difficile* is considered to be responsible for a spectrum of diseases ranging from asymptomatic colonization to diarrhea of varying severity to life-threatening pseudomembranous colitis. Diseases that it causes are known as *C. difficile*-associated diseases (CDAD). Typical manifestations of CDAD are abdominal pain and cramps, profuse diarrhea (mucous, greenish, watery, foul-smelling stool), with fever and leukocytosis (10). A case of CDI is defined by the presence of symptoms (usually diarrhea) and either a positive stool test for CDI toxins, polymerase chain reaction (PCR) or colonoscopic findings revealing the presence of pseudomembranous colitis (2, 5).

Briefly, according to the definition of guidelines for CDI treatment by ESCMID, CDI is divided into hospital cases (those that occurred in hospitals, or nursing homes 48 hours after admission, or within 4 weeks after discharge from these institutions); and outpatient cases (those that occurred outside hospital, before hospital admission, and the patient has not been hospitalized in the past 12 weeks) (5). The increase in CDI mortality rate which has been recorded since 2000 is associated with the emergence of hypervirulent strain of *C. difficile* ribotype 027 which is characterized by much stronger production of toxins A and B, resistance to fluoroquinolones and production of a binary toxin (18). It is also unfavorable that a large number of adults at-risk no longer present typical predisposing factors that contribute to CDI development. Additional problems are recurrent CDI infections; 20% of those who recover from the first CDI infection will suffer again (18).

We have shown that CDI is indeed present in our setting and it was the leading cause of healthcare-associated diarrhea with sometimes fatal consequences. From a total of 347 patients admitted and treated for CDI, we were able to collect data for 276/347 (79.5%) of them, but due to technical and financial constraints molecular diagnosis of CDI was not performed. However, these data are from a hospital center with limited resources and budget, and are a true representation of our condition. Thus, our research has shown that the mean incidence of CDI at UCC Tuzla was 2.23 per 10,000 inpatient days, similar to numbers reported by Barbut *et al.* (19). These authors reviewed the mean incidence of healthcare-associated CDI in 23 European hospitals and reported European CDI average of 2.45 per 10,000 inpatient days (range 0.1-7.1). This is slightly lower than the incidence reported by Bauer *et al.* (4) with a mean incidence of healthcare-associated CDI in 34 European hospitals of 4.1 per 10,000 inpatient days (range 0.0-36.3). In several departments at UCC Tuzla, the mean incidence of healthcare-associated CDI was substantially higher; e.g. the Clinic of Orthopedics and Traumatology, 8.4 per 10,000 inpatient days. The reasons are many, including a somewhat injudicious use of antibiotics and poor on-ward prophylaxis.

It was shown that the annual rates of CDI hospitalization were 15.68 per 10,000 admissions at UCC Tuzla, in conjunction with a rise in annual rates since 2009 to 2012. The increasing rate of CDI hospitalizations in hospitalized patients has been also reported by other studies (9, 20-22). Most of our patients fit the previously determined risk profile such as age over 65, presence of severe comorbidity (heart disease, lung disease, immunodeficiency, previous surgical intervention), and most of them were receiving antibiotics within 3 months, or within 1 month prior to infection; usually cephalosporines, quinolones or different combinations of antibiotics (1, 2, 4, 10). Most studies have shown that the vast majority of patients with CDI had previously taken antimicrobial drugs, which is practically a *conditio sine qua non* for the empirical diagnosis (2, 23). Wide use of antimicrobials and tendency to polypragmasia implies that accurate quantification of CDI risk associated with a particular antibiotic is very difficult. A number of applied antimicrobials, a larger number of doses and longer duration of administration were associated with an increased risk for CDI.

Our research has shown that almost a half of our patients with CDI had previous surgery within 3 months and not only gastrointestinal but also other types of surgeries, which is different from other reports where gastrointestinal surgery was the prominent risk factor for CDI (1). However, most of these patients also had other risk factors for CDI.

A relatively small ratio of patients (7.25%) was infected with *C. difficile* in the outpatient setting. This is somewhat lower than in other reports which reported 11-28% patients with community-associated CDI (12, 24, 25). Whether this is due to lower exposure to *C. difficile* in the community or a lower incidence of antibiotic therapy predisposing for CDI remains to be explored.

In historical terms, the mortality of CDI is low, whether it is a direct or indirect result of the infection, and its value is less than 2% (2). The increase in deaths attributed to CDI, which has been recorded since 2000, is associated with the emergence of hypervirulent strain of *C. difficile* ribotype 027 which is characterized by much stronger production of toxins A and B,

the resistance to fluoroquinolones and production of a binary toxin (26). In our study out of all patients who died, in 11/41 (26.82%) deaths were CDI-associated. These results were similar to the study by Bauer *et al.* (4), where the mortality rate caused by hypervirulent strain of *C. difficile* at three-month follow-up in 34 European hospitals was 22%, and direct mortality from CDI was 40%. Technical inability to perform molecular diagnosis of CDI hampered a detailed analysis of the cause of death of our patients. All 11 patients in our study, whose death was a direct result of CDI, had similar risk factors (over 70 years old, healthcare-associated infection, comorbidity), similar to other studies (2, 4, 5).

Complicated CDI were reported in 1/5 patients, and recurrent infections in ¼, as in other reports (2, 4, 12, 17). Recurrent CDI are a particular problem because besides additional costs to the healthcare system due to repeated hospitalizations and increasing costs of treatment, they also are ongoing frustration for patients. The treatment of our CDI patients was guided by relevant guidelines: Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults: 2010 Update by the Society for Healthcare Epidemiology of America (SHEA) and Infectious Diseases Society of America (IDSA) and ESCMID's algorithm for the treatment (2, 5).

## 5. CONCLUSION

CDI in our hospital center is a much bigger problem than it was thought, although probably not only in our hospital but rather throughout Bosnia and Herzegovina. This research has important practical significance as it will help us to be more aware that the CDI is widely present in our setting. We have to give a serious thought to this clinical problem and more efficiently carry out the diagnosis, treatment, control and prevention of this infection. Our data also point to the importance and necessity of introducing molecular diagnostics of this infection, because prevalence data on PCR ribotypes in European and hospitals worldwide indicate the need for international monitoring of detection, treatment and control of CDI.

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