Update of Risk Factors on *Clostridium difficile* Infection amongst Orthopaedic Trauma Patients in a High Volume Tertiary Care Emergency Center

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Abstract

The presence of *Clostridium difficile* in the human digestive system may manifest itself as asymptomatic colonization, or incidences of diarrhea with different levels of clinical severity or pseudomembranous colitis. The onset of *Clostridium difficile* infection is most often associated with prolonged hospital stay or broad-spectrum antibiotic use during hospitalization. This open prospective cohort study comprising 47 out of 1627 hospitalized patients for whom the presence of diarrhea was reported and thus were suspected for *Clostridium difficile* infection. The diagnosis of the CDI was based on clinical and microbiological findings. The incidence of infections caused by CDI in our study was 2.75/1000 patient days. *Clostridium difficile* infection was eventually proven in 27 out of the 47 patients with reported diarrhea, which represents almost 60% of the entire cohort. Univariate analysis of risk factors related to CDI showed that the infection depended on age, number of hospitalization days and length of use of cephalosporins, while multivariate analysis showed that the only independent risk factor for CDI was age over 50. Careful and limited use of cephalosporin antibiotics, especially in patients over the age of 50, should be implemented in the antibiotic stewardship policy.

Keywords: *Clostridium difficile* Infection; Risk Factors; Antimicrobial Agents

Introduction

*Clostridium difficile* is a species of anaerobic Gram-positive spore-forming bacteria which can be found in soil, as well as in the colon of the digestive system of animals, healthy children and adults [1]. The presence of CD in the human digestive system may manifest itself as asymptomatic colonization, incidences of diarrhea with different levels of clinical severity or pseudomembranous colitis [2]. *C. difficile* was first isolated in 1935 from the digestive system of a healthy newborn [3,4] and the numerous studies have analyzed the risk factors including age, inflammatory bowel diseases, immunodeficiency, chemotherapy, hypoalbuminemia, antibiotic use, hospitalization [2,5], use of proton pump inhibitors (PPIs), malignant diseases, organ transplantation, etc. [6,7]. The onset of CDI is most often associated with prolonged hospital stay or broad-spectrum antibiotic use during hospitalization [2,8].

Over the past decade, the incidence of infections caused by *C. difficile* has increased dramatically in Europe and North America [9-11], which can be explained by the emergence of CD PCR ribotype 027 hypervirulent strain [12,13].

In the period between 1996 and 2004 in Finland, the number of hospital-acquired CDIs doubled from 16/100000 in 1996 to 34/100000 in 2004 [14]. The European Center for Disease Prevention and Control (ECDC) states that the average rate of hospital-acquired CDIs...
showed a rising trend from 2.45/10,000 patients in 2005 to 4.1/10,000 patients in 2008 [15]. In 2008, the CDI rate was 1/10000 patient days in Czech Republic, in Italy 3.6/10000 patient days, in Spain 4.3 /10000, while incidence rates were higher in the Scandinavian countries [13].

An epidemiological study from 2013, which involved 14 European countries and 37 hospitals, demonstrated that the CDI infection rate ranged from 0.6 - 18.5/10000 patient days, while the median CDI rate was 3.7/10000 patient days. This study revealed great differences in incidence rates in majority of European countries [16].

Recent data from the USA shows that in 2001, 5.6 CDIs were registered per 1000 discharges, while in 2012 this number was 127/1000 discharges [17]. There are scarce reports about CDI in Serbia, but one of them stressed the importance of CDI in Clinical Centre of Serbia (CCS) which affects elderly hospitalized patients with co-morbidities [18].

Apart from that, of particular importance are the data that indicate a change in the epidemiology of CDI and increasing frequency in a population which previously belonged to a low-risk group for the development of CDI, such as children and healthy adults, who did not have risk factors for the onset of infection (hospitalization, application of antibiotics etc) [19].

The Emergency Center (EC) of the CCS, which is a national highest tertiary level centre in a Serbia's healthcare system, is 250-bed Level I trauma and emergency center. It consists of 20 departments, 7 High Care units and 3 Intensive Care Units comprising both medical and surgical clinics. The Department for Orthopaedic Surgery and Traumatology (DOST), part of EC of CCS, provides a treatment for patients with skeletal trauma, spinal trauma and those needed orthopaedic microsurgical interventions, and it is equipped with 46 beds out of which 15 in a two high care units. In DOST more than 1600 patients are treated each year, and about 1450 surgeries are performed per year in two operation theatres. In 2016, 130 polytraumatized patients have been treated in DOST.

Objective of the Study

The objectives of this study were to determine the incidence of infections caused by *Clostridium difficile* amongst patients hospitalized in DOST and to identify the risk factors for the onset of infection caused by that bacterium.

Materials and Methods

This open prospective cohort study was conducted between June 2016 and June 2017, comprising 47 out of 1627 hospitalized patients at the DOST of the EC of the CCS, in Belgrade, for whom was reported the presence of diarrhea and thus were suspected for *C. difficile* infection based on the clinical criteria. By examining the patients personal medical history and collecting all relevant epidemiological information through interviews with medical staff and patients, the following data were registered: age, gender, leading diagnosis, recent hospitalization, transfer from another healthcare facility, initial treatment in intensive care unit, number of hospitalization days before the isolation of *C. difficile*, presence of hospital-acquired infection, antibiotic use and the duration of treatment with specific groups of antibiotics (i.e. cephalosporins, quinolones, aminoglycosides, TMP/SMX, carbapenems, colistin, vancomycin, metronidazole), type of co-morbidities (diabetes, chronic obstructive lung disease, high blood pressure, other diseases of gastro-intestinal or uro-genital system), infections (of bloodstream, urinary tract, surgical site, pneumonia), PPI use and disease (CDI) outcome.

The diagnosis of the CDI was based on clinical and microbiological findings. The infection incidence rates were calculated for 1000 hospital days (as proposed by Centers for Disease Control in Atlanta, Georgia, USA).

All patients in whom diarrhea was registered at admission, as well as those reported with diarrhea in less than 48 hours of hospitalization were excluded from the study.

The study was approved by the internal Institutional Review Board.
Case definitions

According to the protocol adopted in CCS, CDI must meet the following criteria:

1. Positive clinical signs (diarrhea) after 48 hours of admittance.
2. Positive laboratory tests (immunochromatographic test for toxin production and bacteriological culture).

C. difficile identification and isolation

Stool samples were tested within 2 hours of collection and, in case the tests could not be performed rapidly, stored at 4°C until processing. An immunochromatographic test (RIDA QUICK Clostridium difficile Toxin A/B, (R-Biopharm AG, Darmstadt, Germany) was used to diagnose presence of A and/or B toxins. The ethanol shock method was applied to culture C. difficile onto the selective CLO agar (bioMérieux, Marcy l’Etoile, France). After 48 hours of incubation in anaerobic chamber, C. difficile was identified by the characteristic morphology, horse odor, Gram staining and API 20A biochemical test (bioMérieux) or Vitek system with ANC cards.

For accurate diagnosis of CDI, tests that use nucleic acid amplification are recommended. One of them is Xpert C. difficile PCR assay, which is a multiplex real-time PCR that detects the toxin B gene (tcdB), the binary toxin gene (cdt), and the tcdC gene deletion at nt 117. The Xpert C. difficile PCR (Xpert PCR) was performed according to the manufacturer’s instructions. Briefly, a stool sample was collected on a swab (Cepheid collection device) from the container received in the laboratory and transferred into the sample reagent vial. The vial was vortexed for 10 s and the solution pipetted into the chamber of the cartridge by using a Pasteur pipette. The cartridge was then placed on the GeneXpert instrument, and the test was performed using the GeneXpert C. difficile assay program. Obtained results can include the following: toxigenic C. difficile positive/presumptive 027-NAP1-BI negative, toxigenic C. difficile positive/presumptive 027-NAP1-BI positive, toxigenic C. difficile negative/presumptive 027-NAP1-BI negative, invalid, error, or no results.

Apart from C. difficile, stool samples were screened for other bacterial pathogens that could cause enterocolitis (Salmonella, Shigella, Yersinia and Campylobacter spp.) using standard methods of cultivation.

Statistical data analysis

The following methods of descriptive statistics were used in this study: central tendency measures (arithmetic mean), measures of variability (variation interval, standard deviation), and relative numbers. The incidence of infections caused by C. difficile was calculated.

The following methods of analytical statistics were used in this study: empirical distribution identification methods, probability estimation methods (Student’s T-test, chi-square test, Fisher’s exact test), assessing significance of connectivity methods (univariate regression analysis and multivariate regression analysis).

The adequacy of the univariate model and the significance of independent variables were estimated with probability of $p \leq 0.05$ and the adequacy of the multivariate model with probability of $p \leq 0.1$.

Statistical data analysis was performed in SPSS (version 10).

Results

During the study period CDI was eventually proven in 27 out of the 47 patients with reported diarrhea, which represents almost 60% of the entire cohort (Table 1). Based on that, the cohort was divided to 2 groups: patients with confirmed CDI and those in whom CDI could not be confirmed. The incidence of infections caused by CDI in the period June 2016 to June 2017 was 2.75/1000 patient days.

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Out of the total number of patients with diarrhea (n = 47), 16 (34%) were male and 31 (66%) were female. The youngest patient was 18, and the oldest was 93 (median age 66.6 ± 18). These and other demographic and clinical characteristics of patients are shown on table 1. Fourteen of them were admitted to the intensive care unit. Univariate analysis shows that significantly more patients in CDI group were over 50 years of age (p = 0.029) and that they stayed in hospital longer (39 days) than members of non-CDI group (25.8 days, p = 0.021).

The number of patients transferred from another healthcare institution to the DOST, as well as the number of patients with previous hospitalization, was almost equally distributed among both groups of subjects. Comparison of distribution of orthopaedic diagnoses, comorbidities and various infections, proton pump inhibitors use (omeprazole, lansoprazole, rabeprazole) and death rate was revealed not to be statistically significant between the two compared groups (Table 1).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number of patients</th>
<th>C. difficile infected</th>
<th>C. difficile non-infected</th>
<th>RR (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>16 (34)</td>
<td>9 (33,3)</td>
<td>7 (35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>31 (66)</td>
<td>18 (66,6)</td>
<td>13 (65)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td>1,219-37,155</td>
<td>0,029*</td>
</tr>
<tr>
<td>&lt; 50</td>
<td>2 (7,4)</td>
<td>7 (35)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 50</td>
<td>25 (92)</td>
<td>13 (65)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
<td>0,518-1,457</td>
<td>0,908</td>
</tr>
<tr>
<td>Polytrauma</td>
<td>9 (19,1)</td>
<td>4 (14,8)</td>
<td>5 (25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip fractures</td>
<td>29 (61,9)</td>
<td>18 (66,7)</td>
<td>11 (55)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Humeral fractures</td>
<td>3 (6,4)</td>
<td>1 (3,7)</td>
<td>2 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibial fractures</td>
<td>3 (6,4)</td>
<td>3 (11,1)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3 (6,4)</td>
<td>1 (3,7)</td>
<td>2 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transfer from other medical institution</td>
<td>7 (14,9)</td>
<td>4 (14,8)</td>
<td>3 (15)</td>
<td>0,194-4,994</td>
<td>0,986</td>
</tr>
<tr>
<td>Previous hospitalizations</td>
<td>10 (21,2)</td>
<td>6 (22,2)</td>
<td>4 (20)</td>
<td>0,257-3,245</td>
<td>0,925</td>
</tr>
<tr>
<td>No of hospitalization days (mean/± SD)</td>
<td>39/± 3,52</td>
<td>25,8 ± 3,09</td>
<td></td>
<td>1,009-4,994</td>
<td>0,021*</td>
</tr>
<tr>
<td>Comorbidities</td>
<td>17 (63)</td>
<td>13 (65)</td>
<td></td>
<td>0,274-3,057</td>
<td>0,275</td>
</tr>
<tr>
<td>Proton pump inhibitor use</td>
<td>18 (66,7)</td>
<td>15 (75)</td>
<td></td>
<td>0,183-2,422</td>
<td>0,667</td>
</tr>
<tr>
<td>Infection (SSIa, UTIb, BSIc, pneumonia)</td>
<td>13 (48)</td>
<td>11 (58)</td>
<td></td>
<td>0,413-4,193</td>
<td>0,642</td>
</tr>
<tr>
<td>Outcome (lethal)</td>
<td>6 (22,2)</td>
<td>4 (20)</td>
<td></td>
<td>0,257-3,245</td>
<td>0,925</td>
</tr>
</tbody>
</table>

Table 1: Characteristics of C. difficile infected and C. difficile non-infected patients with diarrhea.

*: Surgical Site Infection; b: Urinary Tract Infection; c: Blood Stream Infection; *: Statistically significant.

C. difficile grew from stool cultures of 27 patients and they made CDI group while in 20 patients growth could not be obtained and those patients were members of non-CDI group. Immunochromatography tests from stools of C. difficile carriers showed positive results on toxin A or B or both for all members of CDI group. Due to the supply constraints, real time PCR tests could not be applied to all 27 stool specimens of CDI group patients, but to 17 of them (63%). The results confirmed toxigenic C. difficile positive/presumptive 027-NAP1-BI.
positive in 16 out of 17 tested stools (94.1%), which was 59.3% of the entire CDI group. One stool tested toxigenic \textit{C. difficile} positive/presumptive 027-NAP1-BI negative. Other causative agents of enterocolitis were not confirmed in both groups.

All patients from the study received at least 1 antimicrobial agent. The univariate analysis did not demonstrate a significant difference in the prevalence of particular antibiotics usage (Table 2). There was no difference between two patient groups considering the duration of antimicrobial agents use except for cephalosporines (p = 0.049) (Table 2).

### Table 2: Incidence and length of use of antibiotics in the groups of \textit{C. difficile} infected and \textit{C. difficile} non-infected patients - univariate analysis.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Incidence of CDI</th>
<th>OR 95% CI</th>
<th>p-value</th>
<th>Length of use of antibiotics prior to CDI</th>
<th>OR 95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalosporins</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>19 (70.4)</td>
<td>9 (45.0)</td>
<td>0.868-9.712</td>
<td>0.080</td>
<td>6.22/7.11</td>
<td>2.62/4.14</td>
</tr>
<tr>
<td>Quinolones</td>
<td>8 (29.6)</td>
<td>5 (25)</td>
<td>0.342-4.665</td>
<td>0.886</td>
<td>2.53/5.07</td>
<td>2.05/4.29</td>
</tr>
<tr>
<td>Amino-glycosides</td>
<td>10 (37)</td>
<td>6 (30)</td>
<td>0.399-4.718</td>
<td>0.625</td>
<td>2.81/6.16</td>
<td>2.20/4.17</td>
</tr>
<tr>
<td>TMP/SMX</td>
<td>1 (3.7)</td>
<td>2 (10)</td>
<td>0.029-4.111</td>
<td>9.401</td>
<td>0.33/1.73</td>
<td>1.30/4.76</td>
</tr>
<tr>
<td>Carbapenems</td>
<td>2 (7.4)</td>
<td>3 (15)</td>
<td>0.068-3.008</td>
<td>0.413</td>
<td>0.44/1.69</td>
<td>0.50/1.60</td>
</tr>
<tr>
<td>Colistin</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>0.000-0.987</td>
<td>1.000</td>
<td>0.29/1.53</td>
<td>0.25/1.11</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>10 (37)</td>
<td>7 (35)</td>
<td>0.437-5.418</td>
<td>0.502</td>
<td>4.22/7.02</td>
<td>3.20/7.36</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>20 (74)</td>
<td>13 (65)</td>
<td>0.183-2.442</td>
<td>0.538</td>
<td>5.62/6.11</td>
<td>6.11/6.04</td>
</tr>
</tbody>
</table>

Patients’ age, number of hospitalization days, cephalosporin use and duration of that use were subjected to multivariate analysis, which pinpointed to age of patients over 50 years (p = 0.036) as significant contributor to CDI occurrence (Table 3). The multivariate analysis included all p-values greater than or equal to 0.01 (≥ 0.01): age (p = 0.029); number of hospitalization days (p = 0.021); cephalosporin use (p = 0.080), and cephalosporin use duration (p = 0.049).

### Table 3: Independent risk factors of infection with \textit{C. difficile}-multivariate analysis.

<table>
<thead>
<tr>
<th></th>
<th>B*</th>
<th>SE*</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of patients</td>
<td>2.173</td>
<td>1.038</td>
<td>1.148-67.154</td>
<td>0.036*</td>
</tr>
<tr>
<td>No of hospitalization days</td>
<td>0.056</td>
<td>0.030</td>
<td>0.998-1.121</td>
<td>0.056</td>
</tr>
<tr>
<td>Use of cephalosporins</td>
<td>1.250</td>
<td>0.960</td>
<td>0.526-23.179</td>
<td>0.195</td>
</tr>
<tr>
<td>Length of cephalosporins’ use</td>
<td>0.006</td>
<td>0.093</td>
<td>0.839-1.207</td>
<td>0.945</td>
</tr>
</tbody>
</table>

The present study analyzed the risk factors and the incidence of hospital acquired infections caused by \textit{C. difficile} amongst orthopaedic trauma patients in the highest tertiary level emergency institution in Serbia’s health care system. Patients with symptoms of enterocolitis were divided into 2 groups: CDI group, with microbiological confirmation of the infection, and non-CDI group, with clinical signs of enterocolitis, but without the confirmation of \textit{C. difficile} in stools.

### Discussion

In the CDI group, the duration of the third generation cephalosporin’s use was 4.39 days whilst in the group without CDI it took 2.51 days, which was markedly different (p = 0.0001).

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The present study revealed a significant difference between the observed groups regarding the age of patients. CDI was significantly more frequent in the group of patients over the age of 50 (p = 0.029) and this group of patients was 1.2 times more likely to develop an infection in comparison to the non-CDI group (Table 1). Although some other researchers got to similar results [20,21], a study carried on in another healthcare institution in Belgrade [22] involving 200 patients, did not show any correlation between age and the occurrence of CDI. But, Belgrade study took into consideration all surgical patients, and the diseases they suffered from could not be completely comparable to the diseases from the DOST. All patients at the DOST, either from CDI or non-CDI group, were treated for fractures and traumas and were more homogenous population than the group of patients who underwent general surgery. General surgery is the department where most of the patients are treated for various surgical diseases; they can acquire CDI, but the most severe CDI acquired elsewhere are also treated operatively at that department (colectomy because of toxic megacolon, fulminant colitis, septic shock) and it could affect the results of epidemiologic investigations and make the difference between the results of investigations on DOST and other surgical departments.

Hospitalization length is one of the most significant risk factors for CDI [23-25], especially if longer than 15 days [8,26,27]; the present study confirmed it, since the average number of hospital days from admission to discharge from the hospital was 39 in CDI group and 25.8 days in non-CDI group (p = 0.021) (Table 2). C. difficile is a nosocomial pathogen and prolonged stay in hospital enhances the possibility of acquiring it. When Dubberke [28] analyzed the carriage of C. difficile at admission and discharge from a hospital, he found percentages of 21% and 24% carriers respectively. However, the number of carriers of hypervirulent ribotype 027 doubled at discharge, for unclear reasons, and that ribotype is capable of causing the most severe infections [29]. Culture proven C. difficile in 27 patients from CDI group in this study were toxin A and/or B positive, while PCR tests confirmed toxin production in all analyzed stools. The PCR testing was applied to only 63% of samples, but results are pretty convincing about the prevalence of presumptive 027 ribotype in DOST patients, since it was verified in more than a half (16 or 59.3%) of the specimens of the entire CDI group. The research carried on in CCS from 2011 - 2013 [30], encompassing 3 isolates of C. difficile obtained from patients from DOST, revealed that all of them were of PCR ribotype 027. Moreover, it confirmed predominance of 027 (88.54%) in two healthcare institutions in Belgrade, one of which was CCS (88.09% strains typed were of 027 PCR ribotype).

Although longer hospitalization confers the risk of acquiring CDI, previous hospitalization does not the number of patients transferred from some other healthcare facility to DOST as well as the number of patients hospitalized previously was almost equally distributed in both groups (CDI and non-CDI), implying that different factors were important for acquisition of CDI in this facility.

The use of PPIs may lead to acquisition of CDI [7,21,22,25,31,32], but the analysis of our cohort did not reveal the difference between patients with proven CDI and non-CDI group pertaining the use of PPIs (p = 0.667). It seems that more probable cause of enterocolitis that did not occur due to C. difficile might be the disturbance of bowel flora due to use of antibiotics, since all patients from the study received at least 1 antimicrobial agent. While the use of antibiotics as well as the length of their usage have been proven to be amongst leading risk factors for CDI [2,8,21,22,25,31,33,34], the univariate analysis did not reveal a statistically significant difference in the prevalence of the use of any particular antibiotic groups nor in the duration of antibiotic therapy between the CDI and non-CDI patients. However, unlike other antibiotic groups, the mean number of days of cephalosporin use in the CDI patient group was 6.22 comparing to 2.62 in the non-CDI group, which was a statistically significant difference (p = 0.049). Moreover, in the CDI group, the duration of the third generation cephalosporin's use was significantly longer than in the group without CDI (p = 0.0001). The association between cephalosporin use and C. difficile overgrowth is so well established [21,22,32] that there is an opinion that cephalosporins should not be prescribed in medication of the elderly patients [35]. In clinical practice, restriction of cephalosporin use has resulted in a decrease in the numbers of patients with C. difficile [36] and that effect should be kept on mind when establishing guidelines in the future antibiotic stewardship policy of DOST.

The patients with CDI were not at greater risk for death than patients without that infection (p = 0.925) (Table 1). Hospital deaths are more frequent in the populations with other risk factors for CDI, like general surgery patients [21,22,32]. Small number of fatal outcomes was also noticed in another study carried on in CCS, at Clinic for Infectious and Tropical Diseases [18] and we conclude that at least in

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DOST, CDI rarely leads to death. However, CDI is a common infection at the DOST, for incidence of 2.75/1000 patient days, compared to European average of 3.7/10000 patient days [16].

Conclusion

Univariate analysis of risk factors related to CDI revealed that infection in patients of DOST depended on age, number of hospitalization days and length of use of cephalosporins, while by multivariate analysis the only independent risk factor for CDI was age over 50. Although CDI is a serious infection, it did not affect mortality in patients. To achieve the appropriate prevention and management and to lower the rates of CDI in DOST, careful and limited use of cephalosporin antibiotics, especially of third generation, in patients over the age of 50, should be implemented in the antibiotic stewardship policy.

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