

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Epidemiological characteristics of infections caused by bacteria *Clostridioides difficile* toxins

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Introduction/Objective *Clostridioides difficile* is one of the most common infectious agents and an important cause of infections among hospitalized patients, often resulting in severe and potentially fatal outcomes.

The objective of this study was to determine demographical characteristics (age and sex distribution) and outcomes among hospitalized patients with *Clostridioides difficile* infection, and to analyze differences in toxin A, toxin B or toxin A/B prevalence among hospitalized patients with *Clostridioides difficile* infection.

Methods Retrospective descriptive analysis of 200 patients hospitalized at the Institute for Pulmonary Diseases of Vojvodina, Serbia, from 2015 to 2018 was performed. The data were obtained using a standardized "Active surveillance of *Clostridioides difficile*" questionnaire. A non-parametric χ^2 test and binomial logistic regression was used to validate all hypotheses: focusing on higher infection rates and mortality in the elderly compared to younger populations, and the predominance of diagnostic methods isolating both toxins A and B.

Results There are statistically significant differences in the distribution of infection cases among age groups, particularly with a higher prevalence in individuals aged 66 and older, ($p < 0.001$). There is a statistically significant difference in the frequency of respondents in relation to the detection of toxins. Percentage of representation of toxins is 61.5%.

Conclusion The results show that the most common diagnostic method is the detection of toxins A and B, rather than isolating either toxin independently. However, the study suggests that certain diagnostic methods should be supplemented by other newer diagnostic methods.

Keywords: *Clostridioides difficile* infections; hospital infections; preventive measures

INTRODUCTION

Clostridioides difficile (*C. difficile*) represents a significant public health issue exacerbated by the widespread use of antibiotics. Although it is an anaerobic gram-positive bacterium that is found both in the intestinal flora and soil, it also poses a significant risk of infection among both healthy individuals and hospitalized patients [1]. In the United States of America, around half a million new infections are reported each year, while approximately 14,000 deaths are caused by this pathogen [2]. Upon entering the gastrointestinal tract, *C. difficile* transitions from a spore form to an active vegetative state, which leads to the appearance of infection. What makes this bacterium particularly dangerous are the toxins it secretes, namely toxin A and toxin B. Toxin A enhances the cytotoxic effect of toxin B. These toxins synergistically destroy intestinal epithelial cells and significantly disrupt the intestinal barrier [3]. It is believed that asymptomatic colonization of patients admitted to the health care facility shows a prevalence rate ranging from 0.6% to 13% [4]. Today, three types of antibiotics are most often used in the treatment of this infection: vancomycin, metronidazole,

and fidaxomicin. Fidaxomicin has been proved to be the most effective in managing recurrent infection [4]. Resistance to these treatments often leads to pseudomembranous colitis, characterized by severe intestinal damage, diarrhea, and potentially fatal outcomes [5, 6]. Certain studies show the key role of disrupted intestinal microbiota in facilitating *C. difficile* growth. In addition to the bacterial microflora of the intestine, it is important to emphasize that the disturbed fungal microflora also leads to a significant worsening of the clinical picture in people infected with this bacterium [7]. Besides causing pseudomembranous colitis in humans, this bacterium also exhibits pathogenicity in various animal species causing similar disease profiles. However, bacteriophage therapy offers a targeted alternative, leveraging virus specificity against bacterial strains to effectively mitigate infection [8]. Moreover, in the case of the bacterium *C. difficile*, it was discovered that plasmids can affect both pathogenic potential and antibiotic susceptibility, impacting the regulation and production of its toxins. The research objectives of these studies were to detect a potential change in the genome of this bacterium that would lead to increased sensitivity of *C. difficile* to antibiotics [9].

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METHODS

Patients were assessed using a standardized “Active Surveillance of *Clostridium difficile*” questionnaire. Toxin Enzyme Immunoassays (toxin EIA) was used as test to diagnose *C. difficile* infection (CDI). All hospitalized patients were confirmed by toxin EIA of bacteria by isolating toxins A and toxin B, as well as toxins A and B simultaneously. All patients were treated at five clinics of the Institute for Pulmonary Diseases of Vojvodina. The methodological goals included assessing the distribution of CDI across different age groups and sex, two age categories (66 years of age or older compared to 18–65 years of age), assessing the lethality rates among these age categories suffering from the infection, determining the prevalence of toxic detection (either A, B, or both), determining whether there is a statistically significant difference in the frequency of mortality in relation to the specific clinic (1, 2, 3, 4, and 5). Determining whether there is a statistically significant difference in the frequency of respondents by age category in relation to the year of hospitalization. Clinic 1 is the Clinic for Obstructive Pulmonary Diseases and Acute Pneumopathies; clinic 2 is the Clinic for Granulomatous and Interstitial Lung Diseases; clinic 3 is the Clinic of Urgent Pulmonology; clinic 4 is the Clinic of Pulmonary Oncology; clinic 5 is the Clinic of Thoracic Surgery (Table 1).

Table 1. Distribution of outcomes by clinics

Clinics	1	2	3	4	5
Exitus letalis	3	10	22	1	2
Discharged alive	40	49	47	12	14

This table shows the number of people who were discharged alive and who died at the department's clinics

The study was approved by the Ethics Committee of the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia, IRB No (27-III/3).

Statistical analysis

We used the statistical method for data analysis using the non-parametric χ^2 test and binominal logistic regression. This statistical method was utilized to evaluate several hypotheses concerning CDI and outcomes. The hypotheses tested included: increased prevalence of CDI among the elderly compared to younger demographics, higher mortality rates in the elderly population, and more frequent utilization of diagnostic methods detecting both toxins A and B compared to methods detecting only toxin A or toxin B. For the purpose of sample classification outcomes, based on sex and age categories, we use binomial logistic regression. Results were considered statistically significant at $p < 0.05$.

RESULT

This retrospective examination involved the evaluation of 200 hospitalized patients at the Institute for Pulmonary Diseases of Vojvodina from 2015 to 2018. Analysis of data

confirmed the first hypothesis using the χ^2 test, which compared the empirically obtained frequencies against expected frequencies. There are statistically significant differences in the distribution of respondents by age categories, with a higher representation in the age category of 66 years and older, ($p < 0.001$). The second hypothesis was evaluated using the χ^2 test. It revealed no statistically significant differences in mortality rates across age categories ($p = 0.55$).

The third hypothesis indicates that there is a statistically significant difference in the frequency of toxin detection among respondents. Significantly more respondents are in the group where both A and B toxins were isolated, compared to groups where only toxin A or only toxin B was isolated. The hypothesis was confirmed at the level of $p < 0.001$. The fourth hypothesis indicates that there is a statistically significant difference in mortality rates depending on the clinic where treatment was received, with the most notable differences observed in Clinic 3 (31.9% mortality rate, and 68.1% discharge rate) at a significance level of $p < 0.05$. The results of data testing for hypothesis five showed that there is no statistically significant difference at the $p < 0.05$ level in the frequency of patients by age category in relation to the year of hospitalization ($p = 0.33$). Examining the interaction of sex and age category in the context of lethality was performed by binomial logistic regression. The indicator of significance of the logistic regression is the χ^2 test. Sex and age do not make statistically significant contributions to determining whether an individual belongs to the lethality category (*exitus letalis*/discharged).

DISCUSSION

The main characteristic is its multidrug resistance, including resistance to carbapenems. Clinically, CDI often presents with hematochezia, typically associated with significant dysbiosis of the human intestinal microbiota. This dysbiosis exacerbates the clinical manifestations of the infection [10, 11]. Moreover, one of the effective methods of protection and treatment against various pathogens is microbiome refining, offering a safer and more efficacious alternative to fecal microbiota transplantation [12]. In a case report study, we can see the ability of this bacterium to cause emphysematous cystitis [13]. Probiotics are increasingly recognized as an effective intervention for various diseases, with an emphasis placed on the treatment of intestinal infections. Probiotics represent bacteria that are integral to the normal intestinal microflora of an organism [14]. Certain studies have shown that prolonged use of proton pump inhibitors can disrupt this microflora by suppressing hydrochloric acid secretion in the stomach. In such patients, it would be desirable to use probiotics to prevent intestinal infections, including those caused by the bacterium *C. difficile* [15]. Both in vitro and murine studies have highlighted the role of bile acids, which, due to various biochemical processes, slow down and prevent the growth and development of this bacterium [16]. Certain

studies have shown that a mixture of different types of antibiotics have a statistical significance in the prevention and reduction of diarrhea, as well as infections caused by the bacteria *C. difficile* [17]. There is always the possibility of false negative test results for *C. difficile*. In a study conducted over 15 months in an acute care facility, 50 out of 2308 samples tested showed an inverse correlation between negative polymerase chain reaction (PCR) results and positive stool cultures for toxigenic *C. difficile* detection of this bacterium due to discordant samples led to different ribotyping patterns indicating that they originated from different strains. In most cases, false-negative *C. difficile* test results did not appear to affect clinical outcome in these patients. The PCR detection limit can affect the results of molecular methods for the detection of this bacterium [18]. In a single study, a total of 17 isolates of *C. difficile* from garden soil and shoe soles in Perth, Western Australia, failed to grow as black colonies on ChromID agar. MALDI-TOF MS analysis confirmed that these strains are *C. difficile* bacteria. These white colonies of *C. difficile* bacteria from samples and the environment, potentially overlooked when using ChromID bacteria *C. difficile* agar, present no pathogenic threat but highlight risks of false-negative results [19]. There are three leading methods for identifying a toxigenic strain of *C. difficile*: toxigenic culture, a two-step method that combines *C. difficile* culture, cell cytotoxicity assay, and enzyme immunoassay for toxin A/B and glutamate dehydrogenase, and nucleic acid amplification assays targeting toxin-encoding genes, including PCR, quantitative PCR, loop-mediated isothermal amplification, and helicase-dependent isothermal amplification of DNA. The method of toxigenic culture is complex and time-consuming, and is mainly used for epidemiological research and evaluation of new methods. The sensitivity and specificity of immunoassays can vary, and must be combined with a specific high-sensitivity approach to compensate for their shortcomings [20]. The leading detection method of toxin A and toxin B represents a rustic but highly valid method. Toxin A significantly increases the secretion of fluid into the intestinal lumen leading to inflammation and damage to the protein structures of the intestine. Toxin B is

responsible for the key cytotoxic effects on the epithelial layer of the digestive tract, but also for the destruction of other cells. At higher concentrations, toxin B can also cause the appearance of blood in the stool. It is believed that toxin A has a greater influence on the gastrointestinal tract. This method of detecting toxins A and B in the stool is one of the fastest and most cost-effective methods for detecting this bacterium [21]. This is also confirmed by our research, which showed that representations are the results that lead to the third hypothesis [22]. In a study conducted in the United States, which examined and followed over 150 million adults, the incidence of CDI was particularly pronounced in hospitalized patients after transplantation [23]. In comparison with our studies, there is a clear correlation between hospitalized patients with CDI and various types of comorbidities, as is the case in our investigations in clinic 3. The mortality rate in our research across the clinics is clearly shown in Table 1. The previous hypothesis is further supported by over 15 studies that were processed through meta-analysis, where individuals with gastrointestinal diseases had comorbidities. However, this study shows the recurrence of CDI in patients with this type of comorbidity [24].

CONCLUSION

In the observed sample of patients, the percentage of deaths was the highest at the Clinic of Urgent Pulmonology, and as a result, CDI poses an increased risk of death for patients in the most critical condition. It is of particular importance in undertaking some preventive measures. Some preventive measures include the therapeutic use of the macrolide antibiotic fidaxomicin. However, *C. difficile* produces strong toxins – toxin A and toxin B, and also leads to the formation of ulcerative colitis posing a severe risk to hospitalized patients with comorbidities. The results also show that the method of isolating toxins A and B is highly reliable for diagnosing this bacterium.

Conflict of interest: None declared.

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Епидемиолошке карактеристике инфекција изазваних токсинима бактерије *Clostridioides difficile*

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САЖЕТАК

Увод/Циљ *Clostridioides difficile* је један од најчешћих инфективних агенаса и важан узрочник инфекција међу хоспитализованим пацијентима, што често резултира тешким и потенцијално фаталним исходима.

Циљ овог истраживања био је да се утврде демографске карактеристике (расподела по полу и старости) и исходи код хоспитализованих пацијената са *Clostridioides difficile*, као и да се анализирају разлике у преваленци токсина А, токсина Б или токсина А/Б међу хоспитализованим пацијентима са инфекцијом бактеријом *Clostridioides difficile*.

Метод Урађена је ретроспективна дескриптивна анализа 200 пацијената хоспитализованих у Институту за плућне болести Војводине, у Србији, у периоду 2015–2018. године. Подаци су добијени применом стандардизованог упитника „Активни надзор над *Clostridioides difficile*“. Непараметарски χ^2 тест и биномална логистичка регресија коришћени су за валидацију свих хипотеза: фокусирање на веће стопе ин-

фекције и морталитет код старијих у поређењу са млађом популацијом, и превласт дијагностичких метода које изолују токсине А и Б.

Резултати Постоје статистички значајне разлике у заступљености испитаника у односу на старосне категорије, при чему се већи број испитаника налази у категорији од 66 година и старијих, на нивоу значајности $p < 0,001$. Постоји статистички значајна разлика у фреквенци испитаника у односу на изолованост токсина. Процент заступљености токсина је 61,5%.

Закључак Наши резултати показују да је чешћа заступљеност дијагностичке методе изолованости токсина А и Б него само токсина А или само токсина Б. Али резултати показују да одређене дијагностичке методе треба да буду поткрепљене осталим новијим методама.

Кључне речи: инфекције бактеријом *Clostridium difficile*; хоспиталне инфекције; превентивне мере