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

Епидемиолошке карактеристике инфекција изазваних токсинима бактерије *Clostridioides difficile*

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## Епидемиолошке карактеристике инфекција изазваних токсинима бактерије *Clostridioides difficile*


**SUMMARY**

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

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

**Methods** Retrospective descriptive analysis of 200 patients hospitalized in the Institute for Pulmonary Diseases of Vojvodina (Serbia) in the period from 2015 to 2018 was performed. The data were obtained using a standardized "Active surveillance of *Clostridioides difficile*" questionnaire. A non-parametric $\chi^2$ test and binominal 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



**САЖЕТАК**

**Увод/Циљ** *Clostridioides difficile* (ЦДИ) је један од најчешћих инфективних агенаса и важан узрочник инфекција, 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 ЦДИ, као и да се анализирају разлике у преваленци токсина А, токсина Б или токсина А/Б међу хоспитализованим пацијентима са инфекцијом ЦДИ.

**Методе** Урађена је ретроспективна дескриптивна анализа пацијената 200 хоспитализованих у Институту за плућне болести Војводине у периоду 2015–2018. године. Подаци су добијени применом стандардизованог упитника „Активни надзор над *Clostridioides difficile*“. Непараметарски $\chi^2$ тест и биномална логистичка регресија коришћени су за валидацију свих хипотеза: фокусирање на веће стопе инфекције и морталитет код старијих у поређењу са млађом популацијом, и превласт дијагностичких метода које изолују и токсине А и Б.

**Резултати** Постоје статистички значајне разлике у заступљености испитаника у односу на старосне категорије, у смеру да 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. Проценат 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:** *Closttridium difficile* инфекције; хоспиталне инфекције; превентивне мере




## 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, approximately 14,000 hospitalized patients safer annually to infections caused by this pathogen, with around half a million new infections reported each year [2]. Upon entering the gastrointestinal tract, *C. difficile* transitions from a spore form to an active vegetative state, which leads to the appearance of an infection. What makes this bacterium particularly dangerous are the toxins it secretes, namely toxin A and 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 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].

**METHODS**

Patients were assessed using a standardized "Active Surveillance of *Clostridium difficile*" questionnaire. Toxin Enzyme Immunoassays (toxin EIA) was used as tests to diagnose (CDI). All hospitalized patients was confirmed by the diagnostic method Toxin Enzyme Immunoassays (EIA) of bacteria by isolating toxins A and B, as well as toxins A and B simultaneously. All patients were assigned to five clinics the Institute for Pulmonary Diseases of Vojvodina. The methodological goals included assessing the distribution of *C. difficile* infection across different age groups and gender, two age cathegories (66 years of age or older compared to 18-65 years of age), assessing lethality rates among these age categories suffering from this 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 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 a clinic for Obstructive Pulmonary Diseases and Acute Pneumopathies, clinic 2 is a Clinic for Granulomatous and interstitial Lung Diseases, clinic 3 is a Urgent Pulmonology Clinic, clinic 4 is a Pulmonary Oncology Clinic and clinic 5 is a Thoracic Surgery Clinic (Table 1).

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 $\chi 2$ testand 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, utilization of the diagnostic method of detection is more frequent of toxins A and B, compared to only toxin A or only toxin B. For the purpose of sample classification outcomes, based on gender and age categories, we use binomial logistic regression. Results were considered statistically significant at $p < 0.05$.

**RESULT**

This retrospective examination of the subjects 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 chi-square 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 chi-square 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 show 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 gender and age category in the context of lethality was performed by binomial logistic regression. The indicator of the significance of the logistic regression is the chi-square test. There are no statistically significant contributions of gender and age in the context of belonging to the lethality category (ex/discharge).



## 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 the 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 *C.difficile bacteria* [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 show that a mixture of different types of antibiotics has 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. deficile.* In a study conducted over 15 months in an acute care facility, 50 out of 2308 samples tested showed an inverse correlation between negative 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 *Clostridium difficile* test results did not appear to affect clinical outcome in these patients. The detection limit of PCR 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 *Clostridium 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 method of detection of Toxin A and Toxin B represents a rustic but highly valid method, which is supported by the observation results shown in Tables 3a and 3b. 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 had comorbidities, but this time gastrointestinal diseases. 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 in the Urgent Pulmonology Clinic, and therefore CDI represents an additional risk for death in the most severe patients. It is of particular importance in undertaking some preventive measures. Some preventive measures include the therapeutic use of the macrolide antibiotic fidaxomicin. However, *C.difficille* produces strong toxins A and 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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**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



 