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# EVALUATION OF CASES OF *CLOSTRIDIOIDES DIFFICILE* DETECTED IN A TERTIARY HOSPITAL IN WESTERN PARANÁ STATE, BRAZIL

# AVALIAÇÃO DE CASOS DE *CLOSTRIDIOIDES DIFFICILE* DETECTADOS EM UM HOSPITAL TERCIÁRIO NO OESTE DO PARANÁ, BRASIL

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## Resumo

Introdução: *Clostridioides difficile* é um bacilo gram-positivo, anaeróbico obrigatório, com esporas subterminales, e é também produtor de enterotoxinas. O uso prolongado de antimicrobianos de amplo espectro em pacientes imunocomprometidos está frequentemente associado ao desenvolvimento de diarreia e colite pseudomembranosa. O objetivo deste estudo foi avaliar os casos de *C. difficile* detectados no Hospital Universitário do Oeste do Paraná (HUOP). Métodos: Os dados foram extraídos do sistema de prontuários eletrônicos do hospital (TASY®), no período de fevereiro de 2018 a janeiro de 2023. Foram analisados dados como sexo, idade, comorbidades, período de manifestação clínica, uso de antimicrobianos, presença de co-infecção por COVID-19, além dos períodos pré-pandemia e pandemia, para verificar se houve mudanças no perfil dos pacientes afetados por complicações. Resultados: Foram avaliados 700 testes para as toxinas A e B de *C. difficile*. A maioria dos pacientes era do sexo feminino e com mais de 60 anos. Conclusão: Apesar do aumento no número de testes realizados nos últimos anos, a pandemia não se mostrou estatisticamente relacionada aos casos.

Palavras-chave: Colite pseudomembranosa; Infecções adquiridas no hospital; Antibióticos; Enterocolite; Diarreia.

## Abstract

Background: *Clostridioides difficile* is an obligate anaerobic, gram-positive bacillus, with subterminal spores. It is also a producer of enterotoxins. Prolonged use of broad-spectrum antimicrobials in immunocompromised patients is often related to the occurrence of diarrhea and pseudomembranous colitis. The study aimed to evaluate the cases of *C. difficile* detected at the Western Paraná University Hospital (HUOP). Methods: Data were collected from the hospital's electronic medical record system (TASY®) from February 2018 to January 2023. We analyzed patient data, such as gender, age, pre-existing pathologies, clinical manifestation period, antimicrobial use, and whether there was COVID-19 co-infection, as well as the pre-pandemic and pandemic periods to determine if there were changes in the profile of patients affected by complications. Results: A total of 700 tests for *C. difficile* toxins A and B were evaluated. Patients were predominantly female over 60 years of age. Conclusion: In addition, it is possible to highlight that, despite the increasing number of tests in recent years, the pandemic has not been statistically related to cases.

Keywords: Pseudomembranous colitis; Hospital-acquired infections; Antibiotics; Enterocolitis; Diarrhea.



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## Introdução

*Clostridioides difficile* is an anaerobic, gram-positive, spore-producing bacterium that produces toxins A and B. It is one of the main microorganisms that cause nosocomial infections and has been associated with high morbidity and mortality rates in recent years[1–3]. Transmission of *C. difficile* occurs mainly through the dissemination of spores by fecal-oral route. It can occur due to contact with asymptomatic carriers, contaminated health professionals, and with both: contaminated hospital instruments and equipment[4].

The main risk factors for infection related to *C. difficile* are immunosuppression, periods of hospitalization longer than seven days, use of broad-spectrum antimicrobials, and age over 65 years. The latter leads to rates about ten times higher than young individuals[5,6].

Some strains of *C. difficile* are more virulent. The main characteristic of such strains is the presence of the NAP1/B1/027 gene, which has the capacity to produce toxin A up to 16 times higher than less virulent strains. Moreover, regarding toxin B, its production is even higher. These strains produce up to 23 times more toxin than less virulent ones[7,8].

The TcdA gene is responsible for the production of toxin A, which is a highly potent enterotoxin. This toxin can cause significant damage to the affected hosts' intestinal mucosa, as well as inflammation and production of fluid secretion. In turn, toxin B, whose production is regulated by the TcdB gene, has a powerful cytotoxic effect. Both toxins contribute to the production of tumor necrosis factor and interleukins, which promote the inflammatory response and can lead to pseudomembranous colitis[9,10].

Laboratory testing for this microorganism is often performed by detecting toxins A and B in fecal samples using a chromatographic immunoassay. Such test can differentially detect each of the toxins in a short period of time, as cultivation in routine laboratories is often impractical[11].

Recently, the potential connection between increased susceptibility to toxigenic gastrointestinal manifestations caused by microorganisms and COVID-19 has been discussed. This occurs because SARS-CoV-2, in addition to affecting the respiratory system, can replicate in the gastrointestinal tract during the infection. Such fact may increase susceptibility to the manifestation of enterotoxic strains of *C. difficile*, which are already present, colonizing the carriers' gut microbiota[12,13].

Due to the significance of *C. difficile* in hospitalized patients, it is critical to assess the presence of A/B toxins in these patients and to discuss the factors that may influence or favor its occurrence. Thus, the objective of this study was to examine the cases of *Clostridioides difficile* detected at the Western Paraná University Hospital (HUOP) between February 2018 and November 2022.

## Methods

### Data collection

The data were collected from the electronic medical record system used at the Western Paraná University Hospital (HUOP), the Philips Tasy ® software, and

the microbiology sector logbook of the HUOP Clinical Analysis laboratory. The investigation was carried out by actively searching the systems as well as by using search filtering for: "microbiological tests" and "*Clostridium difficile* testing". The data collected were gender, age, clinical manifestation period, hospitalization ward, previous use of antimicrobials, and information on the period of hospitalization regarding the coronavirus pandemic (pre-pandemic or pandemic period). We analyzed 700 records of tests for *C. difficile* toxins A/B detected from February 2018 to November 2022 in patients from all HUOP wards.

The project was approved by the Human Research Ethics Committee (CEP) of the Western Paraná State University (UNIOESTE), CAAE No. 64779522.1.0000.0107, under opinion No. 5,804,969.

### Statistical and data analysis

The collected data were organized in Microsoft Excel® spreadsheets and subsequently analyzed using statistical data analysis tools through the methods of descriptive statistics in the IBM® SPSS® software (Statistical Package for the Social Science) edition 27.

The chi-square test ($X^2$) was performed, with a significance level ($\alpha$) of 5% and the number of degrees of freedom according to the formula GL = (L-1) x (C-1), in which the value of "L" corresponds to the number of rows and the value of "C" to the number of columns in the table used to calculate $X^2$. In addition, the p-value was calculated based on the previously determined $X^2$ to assist in evaluating statistical hypotheses, using tabulated p-value = 0.05 as the basis for evaluating the results.

## Results and discussion

A total of 700 tests for *Clostridioides difficile* (CD) toxins A/B were performed on fecal samples during the evaluated period. Out of the tests performed, 9.7% (68 cases) tested positive for one of the toxins, which suggests the possible presence of this microorganism in the patient's gastrointestinal tract. The other 90.3% (632 cases) tested negative for the presence of both toxins in the fecal samples analyzed from the tested patients.

In the study conducted by MARTINEZ et al.[14] in Mexico, the authors observed a 44% incidence of positive CD tests. Out of the 179 patients included in the study, 79 tested positive, with a higher frequency of patients carrying toxin A/B in fecal samples compared to this study.

In the present study, an increase in the frequency of testing for *Clostridioides difficile* toxins was observed in the period evaluated, with an increased rate of 393%, as shown in Figure 1. The number of positive cases followed the increase in the number of tests. When evaluating the frequency of patients with positive results, there was an increase of 1033% between 2018 and 2022.

Considering that testing is not part of any protocol for admission or follow-up of hospitalized patients, it is possible to determine that there has been an increase in testing to consider. Nevertheless, the increase in the number of cases was not statistically significant.

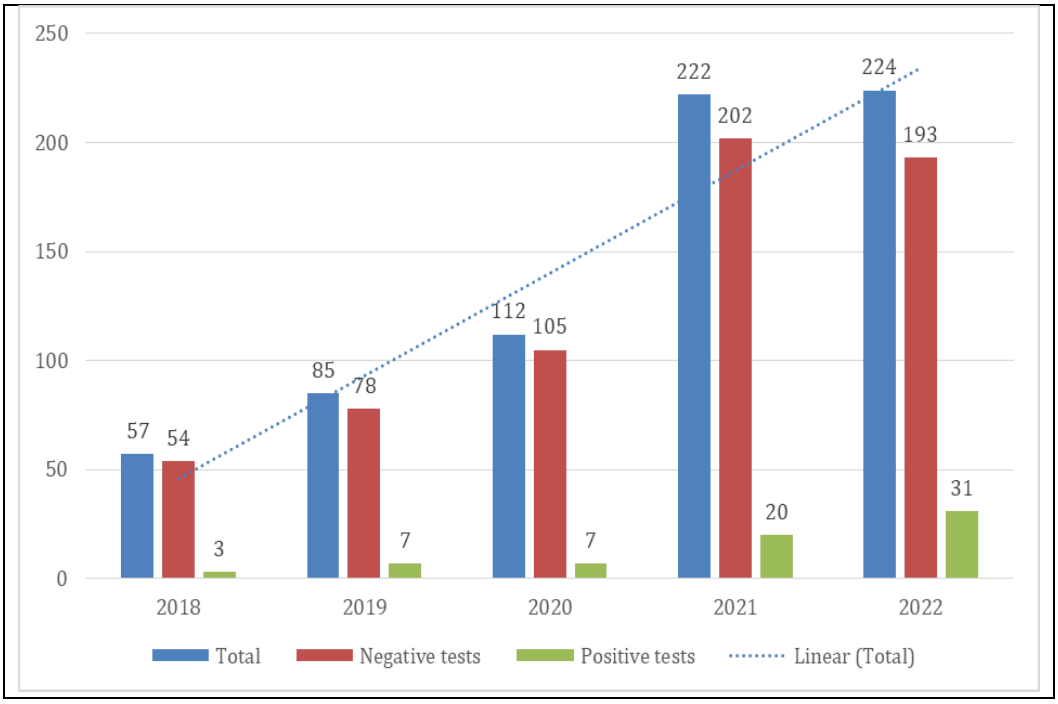


**Figure 1.** Annual increase in the number of tests for *C. difficile* toxins A/B

To adequately characterize the evaluated cases of CD-positive patients, the period of occurrence of the episodes was determined regarding the COVID-19 pandemic (pandemic or pre-pandemic), the hospitalization ward, and patients' genders and ages. This information is summarized in Table 1.

**Evaluation of the relationship between the frequency of positive cases and the pandemic period**

Despite the possible increase in co-infection between COVID-19 and CD, the similarity of symptoms, such as diarrhea, and increased concern over contact precautions with COVID-19 patients may have hindered CD testing, as discussed by Luo et al.[15] This may have led to an under-testing of patients with diarrheal manifestations during the pandemic, resulting in less significant numbers regarding *C. difficile* positive tests in this period, as verified by Otero et al.[16] in their study in a clinical laboratory in Buenos Aires. The authors addressed the impact caused by COVID-19, especially during the first year of the pandemic, which restricted the resources available for the diagnosis and treatment of other diseases.

**Table 1** - Frequency of *C. difficile* cases in the hospital

| Variable | Positive Result | | Negative Result | | Total | | $X^2$ | p-value |
|---|---|---|---|---|---|---|---|---|
| | Freq. | % | Freq. | % | Freq. | % | | |
| **Period of occurrence** | | | | | | | | |
| Pre-pandemic | 11 | 16,2 | 151 | 23,9 | 162 | 23,1 | 2,055 | 0,152 |
| Pandemic | 57 | 83,8 | 481 | 76,1 | 538 | 76,9 | | |
| **Gender** | | | | | | | | |
| Male | 32 | 47,1 | 328 | 51,9 | 360 | 51,4 | 0,576 | 0,448 |
| Female | 36 | 52,9 | 304 | 48,1 | 340 | 48,6 | | |
| **Age** | | | | | | | | |
| Under 2 years old | 2 | 2,9 | 38 | 6,0 | 40 | 5,7 | 6,238 | 0,182 |
| 2 to 18 years old | 7 | 10,3 | 53 | 8,4 | 60 | 8,6 | | |
| 19 to 29 years old | 7 | 10,3 | 38 | 6,0 | 45 | 6,4 | | |
| 30 to 59 years old | 17 | 25,0 | 230 | 36,4 | 247 | 35,3 | | |
| Over 60 years old | 35 | 51,5 | 273 | 43,2 | 308 | 44,0 | | |
| **Hospitalization ward** | | | | | | | | |
| Infirmary | 31 | 31,0 | 178 | 28,1 | 209 | 30,0 | 15,009 | 0,002 |
| ICU | 25 | 37,0 | 400 | 63,3 | 425 | 61,0 | | |
| Emergency Room | 8 | 12,0 | 36 | 5,7 | 44 | 6,0 | | |
| Outpatient clinic | 4 | 6,0 | 18 | 2,9 | 22 | 3,0 | | |

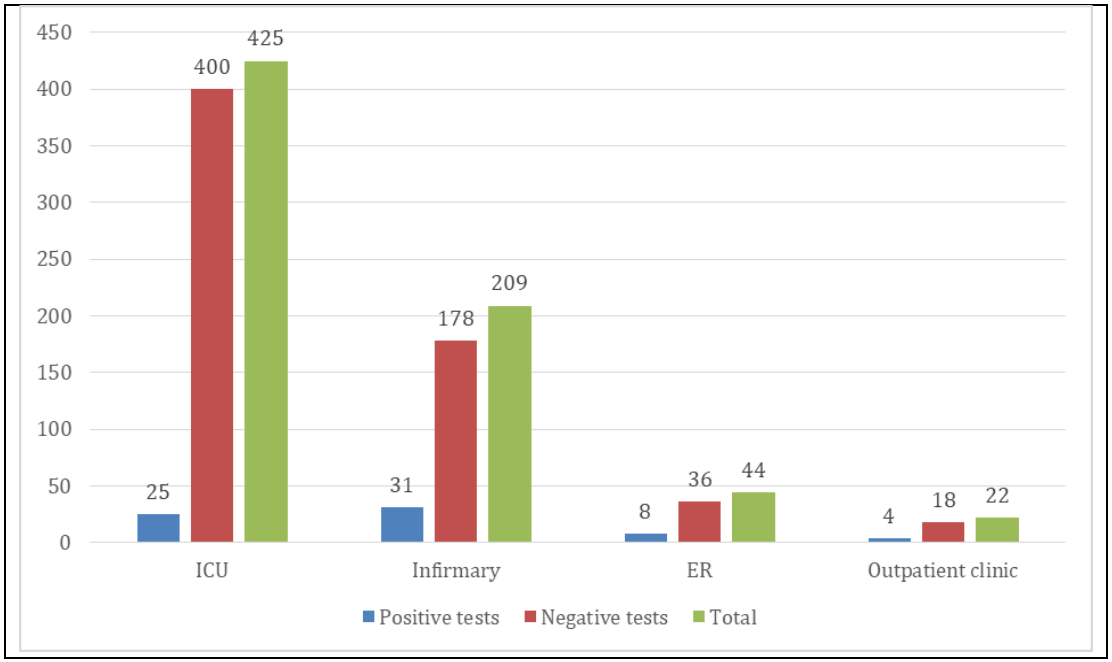


**Figure 2.** Number of tests for *C. difficile* toxins A/B by hospitalization ward.

The chi-square ($X^2$) statistical test was performed to assess the relationship between the frequency of CD-positive cases and the COVID-19 pandemic period. The tabulated value of $X^2$ for 1 degree of freedom and significance level ($\alpha$) of 5% is 3.84[15]; a result below this value, such as the calculated result, indicates independence between the variables evaluated. In the assessment of the p-value, there is not enough evidence to claim that the pandemic period significantly favored the number of positive cases of *C. difficile*.

**Assessment of testing frequency regarding the hospitalization ward**

The frequency of cases was also assessed regarding the patient's hospitalization ward. The ward with the highest number of tests is the Intensive Care Unit (ICU), where the most severe patients requiring advanced monitoring are accommodated, with 425 occurrences. The Infirmary is next with 209 occurrences, of which 74 were in patients accommodated in isolation beds, including those hospitalized for COVID-19. The Emergency Room presented 44 occurrences, and, finally, the Outpatient Clinic, where patients undergoing routine examinations are tested, had 22 occurrences of CD testing. This frequency is demonstrated in the following figure:

Among the cases of positive CD tests, the wards with the highest number of detected cases were the infirmaries which accounted for 31 cases in total. Out of the total of 31 cases, 18 concerned the Isolation Ward that accommodates patients with highly transmissible diseases. The other infirmaries accounted for 13 cases.

Similarly to the observed in the present study, Simmering et al.[17] described that the frequency of occurrence of *C. difficile* is higher among inpatients when compared to the frequency among outpatients.

One of the main factors for the occurrence of diarrhea in hospitalized patients is associated with the use of antibiotics by those colonized by CD. The considerable number of positive cases in the Isolation Ward may be associated with the fact that such patients are often treating bacterial infections and using antibiotics for long periods. Such a fact can cause cases of diarrhea, leading to the investigation of CD colonization through tests for toxins A and B.

Regarding the characteristics of the hospitalization ward and the use of antimicrobials, some studies point to the risk of the patient testing positive for CD in previous antibiotic use. According to Schwartz[18], this risk can increase by up to 22%.

**Assessment of the frequency of positive cases regarding antibiotic use**

Patients who tested positive for CD were assessed for antibiotic use during hospitalization prior to the event that resulted in the diagnosis, covering a previous period of up to 4 weeks. Data regarding the use of antimicrobials by positive patients are described in Table 2.

**Table 2** - Frequency of positive cases for *C. difficile* toxins A/B regarding the use of antibiotic therapy.

| | Frequency | Percentage (%) |
|---|---|---|
| With previous use of antibiotic | 51 | 75,0 |
| Without previous use of antibiotic | 17 | 25,0 |
| Total | 68 | 100,0 |

Tenório et al.[19] evaluated the use of antimicrobials prior to positive testing for *C. difficile*. The authors found that 90.9% of positive patients had been treated with antibiotic therapy prior to the diagnosis. Similarly, Monaghan et al. [20] found in their study that out of the 26 patients positive for toxigenic *C. difficile* strains, 94.4% had been treated with antibiotics previously. A similar result was observed in the present study, in which patients with a history of antibiotic use were the majority of those who tested positive for CD.

### Assessment of the positive patient's profile

Generally, it is possible to verify that CD cases are distributed homogeneously between genders, while the most affected age group is the elderly. Thus, Table 1 presents that the distribution of CD cases between genders is homogeneous, with a slight predominance of females, who account for around 53% of cases, while males account for 47%. Regarding the age group with the highest prevalence, patients over 60 years of age are visibly the most affected, demonstrating predominance in the profile of positive cases for CD in elderly patients, corroborating the findings in the literature.

Jorge et al.'s[21] investigation found a total of 117 positive cases for *C. difficile*. The scholars verified that the predominance of elderly patients with a mean age of 64.1 years and a slight female prevalence of 61.5% was characterized, aligned with the present study's findings.

## Conclusions

The present study has made important contributions to the knowledge and understanding of the epidemiology of Clostridioides difficile infection in a tertiary hospital in western Paraná. Although a statistically significant increase in positive cases has not been observed in recent years, the findings enable the identification of important characteristics regarding the spread and development of such microorganism in a hospital environment.

However, the standardization of testing for the disease still needs to be improved, as well as more representative studies to characterize the risk factors associated with the infection. In addition, it is necessary to deepen the understanding of the epidemiology of C. difficile by exploring information on the classes of antibiotics used and, especially, the length of hospitalization and the profile of pre-existing diseases in infected patients. These efforts are critical to improve prevention and control strategies for C. difficile infection and reduce the impact of the disease on public health

## Conflicts of interest

Authors have no conflicts of interest to report.

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Nothing to report.

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