EVOLUTION OF *CLOSTRIDIOIDES DIFFICILE* INFECTIONS DURING THE COVID-19 PANDEMIC: A RETROSPECTIVE OBSERVATIONAL STUDY AT A TERTIARY UNIVERSITY HOSPITAL

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EVOLUTION OF *CLOSTRIDIOIDES DIFFICILE* INFECTIONS DURING THE COVID-19 PANDEMIC: A RETROSPECTIVE OBSERVATIONAL STUDY AT A TERTIARY UNIVERSITY HOSPITAL (Abstract): *Clostridioides difficile* infection (CDI) is a major cause of healthcare-associated diarrhea. This study aimed to analyze the clinical course and characteristics of patients diagnosed at a tertiary university hospital in northeastern Romania between 2020 and May 2023. **Materials and methods:** This retrospective observational study included 2,317 adult patients with CDI confirmed clinically and microbiologically, colonoscopically, or histopathologically, who were hospitalized between January 2020 and May 2023. Data from medical records were analyzed descriptively using *SPSS 18.0*, employing frequencies, percentages, and measures of central tendency and dispersion. **Results:** The number of CDI cases increased progressively from 2020 to 2022 (rising from 540 to 811), with 290 cases recorded between January and May 2023. Men accounted for 59.6% of the cohort; the mean age was 62.82 ± 15.67 years, with a median age of 65 years. Patients from urban areas represented 57.7% of the total. A combination of toxins A and B was identified in 79.6% of cases. Healthcare-associated CDI accounted for 50.1% of cases, and 50.5% of patients had experienced at least one hospitalization in the preceding year. Recent exposure to acid-suppressive medication was noted in 64.4% of patients, antibiotic therapy in 24.3%, and surgery within the previous two weeks in 13.4%. Immunocompromised patients constituted 55.3% of the group, and recurrence occurred in 9.5% of cases. **Conclusions:** The profile of patients with *Clostridioides difficile* infection was characterized by advanced age, prior hospitalizations, and recent exposure to acid-suppressive medication and antibiotics. The results highlight the need for active surveillance and early identification of high-risk patients to reduce the impact of CDI in the hospital setting. **Keywords:** EPIDEMIOLOGY, *CLOSTRIDIOIDES DIFFICILE*, COVID-19.

INTRODUCTION

Clostridioides difficile infection (CDI) is currently one of the leading causes of healthcare-associated diarrhea, significantly impacting morbidity, mortality, length of hospital stay, and healthcare system costs. Although guidelines regarding CDI awareness, diagnosis, and treatment are available, CDI rates continue to rise in Europe and the United States (1). *Clostridioides difficile* infection is considered the primary cause of healthcare-associated diarrhea in Europe, with a recurrence rate of 15–20% and a mortality rate of 5% (2). Recurrences represent a significant issue in the course of the disease, particularly in older or im-

mune-compromised patients, or in those with repeated exposure to antibiotics and health-care services. In this context, CDI remains a major public health concern, especially within hospital settings. The COVID-19 pandemic, which emerged in late 2019 and was caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), had a major global impact on healthcare systems. Beyond the characteristic respiratory involvement, COVID-19 was also associated with intestinal dysbiosis, and digestive symptoms were reported in a subset of patients (3). While the disease affected all age groups, vulnerable individuals, particularly older adults and patients with pre-existing chronic conditions—were at increased risk of severe disease and adverse outcomes (4).

During the pandemic, hospital activity was affected by the reorganization of patient pathways, the increasing number of critically ill patients, the implementation of strict hygiene measures, patient isolation, and the use of personal protective equipment. These measures may have contributed to reducing the transmission of certain healthcare-associated infections. (5,6). Conversely, the frequent use of empirical therapies, including antibiotics, together with the admission of large numbers of vulnerable patients, maintained concerns regarding CDI trends and outcomes. Furthermore, the overlap between the gastrointestinal symptoms of COVID-19 and those of CDI necessitated heightened attention regarding differential diagnosis and the surveillance of these cases. Prior to the onset of the COVID-19 pandemic, the incidence of CDI was rising in some European countries and in the United States (2).

The study aimed to describe the trends and main characteristics of CDI cases diagnosed between January 2020 and May 2023 at a tertiary university hospital in northeastern Romania.

MATERIALS AND METHODS

Study design

We conducted a retrospective observational study at a tertiary care hospital in northeastern Romania, aiming to provide a descriptive analysis of *Clostridioides difficile* infection cases diagnosed between 2020 and May 2023. The study period was selected against the backdrop of the COVID-19 pandemic, spanning from the beginning of 2020 to May 2023, the point at which the World Health Organization declared the end of the Public Health Emergency of International Concern associated with COVID-19. A total of 2,317 adult patients with a confirmed diagnosis of CDI were included in the analysis. The study's primary objective was to evaluate trends in the number of CDI cases during the analyzed period and to describe the key demographic, epidemiological, clinical, microbiological, and outcome-related characteristics of the patients included in the study cohort.

Study inclusion criteria

The study included patients aged 18 years or older who were hospitalized during the analyzed period, in whom the diagnosis of *Clostridioides difficile* infection was confirmed based on clinical, microbiological, colonoscopic, or histopathological criteria, and for whom medical documentation was sufficient for the collection and interpretation of the data required for the study. The diagnosis of CDI was considered confirmed if at least one of the following criteria was met: the clinical and microbiological criterion, the colonoscopic

criterion, or the histopathological criterion. The clinical and microbiological criterion involved the presence of diarrhea - defined as a minimum of three unformed stools within the preceding 24 hours - or toxic megacolon, associated with a positive stool test for *Clostridioides difficile* toxins A and/or B or a positive PCR test for the genes encoding toxins A and/or B. The colonoscopic criterion was defined by the appearance of pseudomembranous colitis, while the histopathological criterion was defined by the identification of characteristic changes in the biopsy specimen.

Exclusion criteria

Patients under the age of 18, Emergency Department presentations not followed by hospital admission, and cases with incomplete clinical or microbiological data that precluded adequate analysis were excluded.

Data were collected from patients' medical records. For each patient included in the study, relevant demographic and clinical-epidemiological data were recorded - such as age, sex, place of residence, year of diagnosis, type of toxins identified, healthcare-associated infection status, history of hospital admissions within the past year, history of infection recurrence, and immunological status (specifically noting any immunocompromised state) - along with information regarding risk factors associated with CDI.

Statistical analysis

The collected data were statistically analyzed using the *Statistical Package for the Social Sciences (*SPSS) software, *version 18.0*. Given the descriptive nature of the study, qualitative variables were expressed as absolute frequencies and percentages, while quantitative variables were expressed in terms of mean, median, standard deviation, minimum and maximum values, and percentiles.

The statistical analysis aimed to characterize the patient cohort and describe the distribution of cases during the study period.

Scientific research ethics

The study was approved by the hospital's Ethics Committee (No. 105/26.10.2023) and the Research Ethics Committee of UMF Iași (No. 387/30.01.2024). Furthermore, it was conducted in accordance with the ethical principles established by the Declaration of Helsinki.

RESULTS

Between 2020 and May 2023, 2,317 CDI cases were recorded at the tertiary hospital where the study was conducted. The distribution of cases by year showed a progressive increase between 2020 and 2022. In 2020, 540 cases were diagnosed, representing 23.3% of the total cohort. In 2021, 676 cases were recorded (29.2%), and in 2022, the number of cases rose to 811 (35.0% of the total). In 2023, 290 cases were recorded (12.5%), noting that the follow-up period for this year was limited to May (fig. 1).

Regarding sex, male patients predominated, accounting for 59.6% of the study cohort. The male-to-female ratio was approximately 1.5: 1, indicating male predominance within the analyzed cohort (tab. I).

Patient age ranged from 18 to 98 years. The mean age was 62.82 ± 15.67 years, and the median age was 65 years. The 25th, 50th, and 75th percentiles were 52, 65, and 74 years, respectively. The skewness coefficient was -0.494, indicating a mildly negatively skewed age distribution (fig. 2).

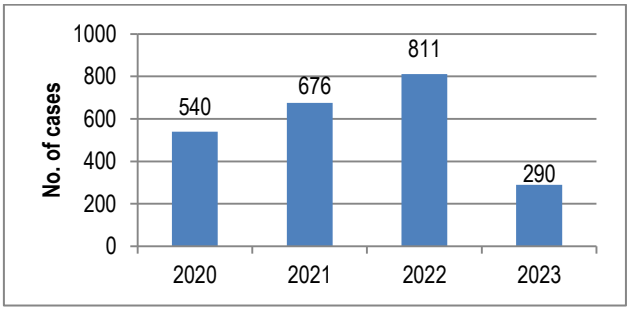

Fig. 1. Number of *Clostridioides difficile* infection cases by study year

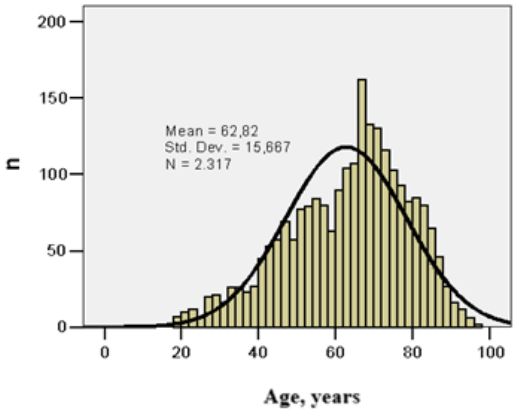

Fig. 2. Age histogram for the study cohort

Regarding the patients' place of residence, 1,337 patients (57.7% of the total) came from urban areas (tab. I).

From a microbiological perspective, toxin A was identified in 330 patients (14.2% of the cohort), and toxin B in 142 patients (6.1%). The concurrent detection of toxins A and B was the most frequent finding, occurring in 1,844 patients and accounting for 79.6% of cases. This distribution highlights the predominance of cases involving the concurrent detection of toxins A and B (fig. 3, tab. I).

Healthcare-associated infection was documented in 1,159 patients, representing 50.1% of the cohort. Additionally, 1,167 patients (50.5%) had at least one hospital admission within the past year (tab. I).

An analysis of treatments administered during the three months preceding CDI symptom onset showed that acid-suppressive medication was used by 1,492 patients, representing 64.4% of the cohort. Recent antibiotic therapy was documented in 562 patients (24.3%), immunosuppressive treatment in 97 patients (4.2%), and cytotoxic therapy in 83 patients (3.6%). Surgical interventions or invasive procedures involving the gastrointestinal tract within the preceding two weeks were reported in 310 patients, representing 13.4% of the cohort. Regarding clinical course, 1,282 patients (55.3%) were classified as immunocompromised. CDI recurrences were documented in 219 patients, corresponding to 9.5% of the cohort (tab. II).

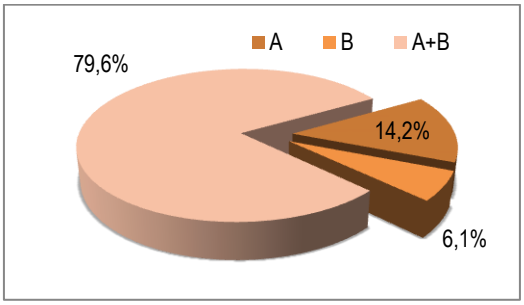

Fig. 3. Composition of the study group by toxin type

TABLE I.

General characteristics of the study group

Characteristics	Cohort (n=2317)
Demographic characteristics	
Diagnosis year 2020 2021 2022 2023	 540 (23.3%) 676 (29.2%) 811 (35.0%) 290 (12.5%)
Male, n (%)	1381 (59.6%)
Age > 65 years, n (%)	1158 (50.0%)
Urban area, n (%)	1337 (57.7%)
Clinical characteristics	
Toxins A B A+B	 330 (14.2%) 142 (6.1%) 1844 (79.6%)
HAI	1159 (50.1%)
Hospitalizations in the last year	1167 (50.5%)

TABLE II.

Therapeutic and clinical-evolutionary factors associated with CDI

Characteristics	Cohort (n=2317)
Treatments in the last 3 months	
Cytotoxic therapy	83 (3.6%)
Immunosuppressants	97 (4.2%)
Acid-suppressive medication	1492 (64.4%)
Antibiotic	562 (24.3%)
Surgical interventions in the last 2 weeks	310 (13.4%)
Clinical course	
Immunocompromised	1282 (55.3%)
Recurrences	219 (9.5%)

DISCUSSION

Our study results highlight a progressive increase in the number of *Clostridioides difficile* infection (CDI) cases between 2020 and 2022 - rising from 540 cases in 2020 to 811 cases in 2022 - followed by 290 cases recorded in 2023, noting that the follow-up period for the latter year was limited to May. This upward trend must be interpreted within the specific context of the pandemic, a period characterized by the reorganization of hospital patient pathways, changes in the profile of hospitalized patients, frequent antibiotic use, and intensified infection control measures.

Existing literature reports inconsistent findings regarding CDI trends during the COVID-19 pandemic (4). Spigaglia notes that data from January 2019 to September 2021 generally indicate a decrease in the incidence of hospital-acquired CDI during the pandemic, a trend potentially explained by intensified hygiene measures, patient isolation, and the use of personal protective equipment (4). However, despite improvements in prevention and control strategies, some single-center studies have observed an increase in hospital-acquired CDI cases during the pandemic (4). For instance, Lewandowski *et al*. observed an increase in CDI rates at a hospital in Warsaw from 2.6% in the pre-pandemic period to 10.9% during the COVID-19 pandemic (7). Consistent with the trend observed in our cohort, Karampatakis *et al*., in a retrospective study conducted at a tertiary hospital in Greece between January 2018 and March 2022, highlighted a significant increase in the incidence of CDI, with a more pronounced rate of increase during the pandemic period compared to the pre-pandemic period (8).

A relevant characteristic of our cohort was the advanced age of the patients. The mean was 62.82 ± 15.67 years and the median was 65 years, indicating that approximately half of the patients were aged 65 years or older. These findings are consistent with the literature, which identifies advanced age as an important risk factor for CDI. A recent systematic review found that age was evaluated in 99 studies, 46 of which included an estimate of risk regarding age as a potential factor for CDI (9). In our cohort, male patients predominated, accounting for 59.6% of total cases. This finding should be interpreted with caution, as the literature does not consistently support male sex as a major independent risk factor for CDI. Conversely, female sex is more frequently reported to be associated with an increased incidence of CDI (10), suggesting that the influence of sex on CDI risk is not uniform and may depend on the characteristics of the study population, the type of exposures, the comorbidity profile, and the clinical context. Thus, the male predominance observed in our study is more likely a descriptive characteristic of the analyzed cohort rather than an indication of a proven causal relationship.

Patients from urban areas accounted for 57.7% of the total. This distribution can be explained by the hospital's location in a major university center and the easier access urban patients have to complex medical services.

From a microbiological perspective, the combination of toxins A and B was the most frequently identified pattern in the analyzed cohort, present in 79.6% of patients. This result aligns with recent literature indicating the predominance of the classic A+B toxigenic profile among *Clostridioides difficile* isolates. For instance, a study conducted across three tertiary hospi-

tals in Xi'an, China, involving 1,231 stool samples from hospitalized patients, isolated 156 *Clostridioides difficile* strains; the tcdA+/tcdB+ profile was the most common, accounting for 78.2% of the isolates (11). Similar figures were reported by Costa *et al*., who identified the tcdA+/tcdB+/cdtB− profile in 80.4% of toxigenic isolates from adult patients with CDI (12). These data confirm that the percentage observed in our study falls within the trends reported in the literature.

Acid-suppressive medication was used by 64.4% of the patients in the study cohort, representing one of the most common potential predisposing factors. This finding is consistent with data from the literature, such as that reported by Cao *et al*. reporting, in a meta-analysis that included 50 observational studies, an increased risk of developing CDI in patients exposed to proton pump inhibitors (PPIs) (13). Therefore, the high frequency of acid-suppressive medication use in our cohort may be considered a marker of vulnerability.

In our study, recent surgical interventions or invasive procedures involving the gastrointestinal tract were reported in 310 patients, representing 13.4% of the cohort. Previous studies have shown that surgical procedures, particularly gastrointestinal interventions, may be associated with an increased risk of postoperative CDI. In an analysis by Columbo *et al.* involving 397,750 patients who underwent surgery between 2015 and 2019, the overall incidence of postoperative CDI was 1.6%, with the highest rates observed following digestive procedures such as small bowel resection and gastrectomy (14). Similarly, Kim *et al*. reported a CDI incidence of 3.6% following ileostomy closure in patients with rectal cancer, identifying adjuvant chemotherapy as an independent factor associated with the occurrence of CDI (15). In the context of our cohort, in which 3.6% of patients received chemotherapy, recent digestive interventions and exposure to chemotherapeutic agents may be considered markers of vulnerability for CDI.

The recurrence rate in our cohort was 9.5%, which was lower than the overall recurrence rates reported in the literature. Guh *et al*. reported that, among 18,829 patients with an initial episode of CDI, 882 (4.7%) developed multiple recurrences, which were more frequently associated with advanced age, recent medical exposure, and prior antibiotic therapy (16). In comparison, Finn *et al*., in a systematic review on the epidemiology of primary and recurrent CDI, indicate that recurrence is commonly estimated at approximately 15–35% of cases, with variations influenced by the definition used, the duration of follow-up, and the profile of the analyzed population (17).

In our study, antibiotic exposure in the three months preceding symptom onset was identified in 24.3% of patients. Antibiotic therapy plays an important role in CDI epidemiology by disrupting the intestinal microbiota and promoting the proliferation of *Clostridioides difficile*. In a retrospective observational study on antibiotic consumption conducted at a multi-unit emergency hospital in Romania, exploratory ecological analyses identified numerically increased temporal covariation between fluoroquinolone consumption and CDI occurrence (18). In our cohort, approximately half of the patients were aged 65 or older. Literature data confirm the heightened vulnerability of older patients to CDI and underscore the need for increased attention toward this demographic within

antibiotic stewardship programs (19).

Regarding prior hospitalizations, 1,167 patients in our cohort, representing 50.5% of the total - had been hospitalized at least once during the year preceding their CDI diagnosis. This high proportion is significant, given that Finn *et al*. identify recent hospitalization, alongside advanced age and antibiotic use, as one of the most frequently reported independent risk factors for an initial CDI episode (17). Similarly, Guh *et al*. reported that prior hospitalization in the 12 weeks preceding the initial episode was significantly more frequent in patients with multiple recurrences compared to those without multiple recurrences - 39.6% versus 26.9%, respectively (16). Thus, although the recurrence rate documented in our study was relatively low, the high frequency of prior hospitalizations supports the existence of a significant vulnerability profile for CDI in the studied population.

CONCLUSIONS

This study provides a relevant overview of the profile of patients with *Clostridioides difficile* infection diagnosed at a tertiary university hospital during the COVID-19 pandemic. The profile of the included patients was characterized by advanced age, prior hospitalizations, exposure to acid-suppressive medication, and recent antibiotic therapy - factors recognized in the literature as significant vulnerabilities for CDI. The results underscore the importance of active CDI surveillance, the rational use of antibiotics, and the early identification of high-risk patients in order to reduce the burden of this condition in the hospital setting.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding regarding this scientific research.

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