Epidemiology of Clostridioides difficile Infection in Portugal: A Retrospective, Observational Study of Hospitalized Patients

Epidemiologia da Infeção por Clostridioides difficile em Portugal: Um Estudo Retrospetivo, Observacional em Doentes Hospitalizados

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ABSTRACT

Introduction: *Clostridioides difficile* is the main cause of healthcare-associated diarrhea in Europe and North America. The aim of this study was to characterize the epidemiology and clinical burden of *Clostridioides difficile* infection among hospitalized patients in Portugal.

Material and Methods: Retrospective study conducted in six public hospital centers in Portugal. All primary *Clostridioides difficile* infection episodes and related recurrences occurring in 2017, as well as episodes developing two to eight weeks after the last episode diagnosed in that year, were documented. The National Reference Laboratory (National Institute of Health Dr. Ricardo Jorge) provided national surveillance data on *Clostridioides difficile* infection.

Results: A total of 385 inpatients with at least one primary episode diagnosed in 2017 were included. Most patients were aged over 70 years-old (73.2%). The included patients developed 451 episodes during the observation period. Approximately 44% of primary episodes were community-associated. Most episodes (94.9%) occurred in patients with one or more risk factors, with recent antibiotic exposure being particularly common (86.0%). All-cause in-hospital mortality was 19.5%, being significantly higher in patients aged over 65 years-old *versus* those aged 18 to 64 years-old (22.4% *vs* 7.8%, respectively). Over 50 different ribotypes were observed among 206 *Clostridioides difficile* strains received by the National Reference Laboratory.

Conclusion: In Portugal, hospitalized patients with *Clostridioides difficile* infection are mostly older patients presenting risk factors for the development of this infection, particularly recent antibiotic exposure. Mortality is disproportionately high among the older population. Community-associated *Clostridioides difficile* infection is common among inpatients with this infection.

Keywords: Clostridioides difficile; Clostridium Infections/epidemiology; Inpatients; Portugal

RESUMO

Introdução: *Clostridioides difficile* é a principal causa de diarreia nosocomial na Europa e América do Norte. Este estudo teve como objetivo caracterizar a epidemiologia e o impacto clínico da infeção por *Clostridioides difficile* em doentes hospitalizados em Portugal.

Material e Métodos: Estudo retrospetivo conduzido em seis centros hospitalares públicos de Portugal. Foram documentados todos os episódios primários de infeção por *Clostridioides difficile* ocorridos em 2017 e consequentes recorrências, bem como os episódios que ocorreram entre duas a oito semanas após o último episódio diagnosticado neste ano. Os dados de vigilância nacional de infeção por *Clostridioides difficile* foram fornecidos pelo laboratório nacional de referência (Instituto Nacional de Saúde Doutor Ricardo Jorge).

Resultados: Foram incluídos 385 doentes hospitalizados com pelo menos um episódio primário diagnosticado em 2017. A maioria dos doentes tinha idade igual ou superior a 70 anos (73,2%). Os doentes incluídos tiveram 451 episódios durante o período de observação. Aproximadamente 44% dos episódios primários eram episódios de infeção por *Clostridioides difficile* adquirida na comunidade. A maioria dos episódios (91,8%) ocorreu em doentes com um ou mais fatores de risco, sendo a exposição recente a antibióticos particularmente comum (86,0%). A mortalidade hospitalar por todas as causas foi de 19,5%, sendo significativamente superior em doentes com idade igual ou superior a 65 anos comparativamente a doentes com idade entre 18 e 64 anos (22,4% *versus* 7,8%, respetivamente). Mais de 50 ribotipos diferentes foram detetados entre as 206 estirpes de *Clostridioides difficile* recebidas pelo laboratório nacional de referência.

Conclusão: Em Portugal, doentes hospitalizados com infeção por *Clostridioides difficile* são, na sua maioria, doentes idosos com fatores de risco para o seu desenvolvimento, particularmente exposição recente a antibióticos. A mortalidade é desproporcionalmente elevada na população idosa. Episódios associados à comunidade são comuns em doentes hospitalizados com esta infeção.

Palavras-chave: Clostridioides difficile; Doentes Internados, Infecções por Clostridium/epidemiologia; Portugal

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INTRODUCTION

Clostridioides difficile (*C. difficile*) is the main cause of healthcare-associated diarrhea in Europe and North America, leading to substantial morbidity and mortality.[1,2] The epidemiology of *C. difficile* infection (CDI) has markedly changed since the turn of the 21st century, with pronounced increases in incidence, severity, and mortality being noted worldwide.[3–5] This epidemiologic shift has been attributed to the emergence of a previously rare strain [hypervirulent ribotype (RT) 027], but which has since been associated with large CDI-related outbreaks of severe disease in Europe and North America.[3,6] In Portugal, the prevalence of this RT was found to be 18.5% among healthcare- and community-associated CDI isolates identified between 2010 and 2015 in 20 acute care hospitals.[7]

Antibiotic exposure is the leading risk factor for CDI development, with nearly all antibiotics being implicated.[5,8] Antibiotics cause a long-lasting disruption of intestinal microbiota, resulting in an increased risk of CDI both during therapy and in the three months after its cessation.[9] Older age (≥ 65 years), hospital admission, recent healthcare exposure, long-term facility residence, chronic diseases (e.g., chronic kidney, lung and heart disease, diabetes, inflammatory bowel disease), immunosuppression, and hemodialysis have also been associated with an increased CDI risk.[5,8,10]

Although historically considered a nosocomial pathogen, an increasing number of CDI cases has been reported in populations without known risk factors, such as antibiotic-naïve patients and those with minimal or no recent exposure to healthcare settings.[4] European and North American data show that an appreciable proportion of CDI episodes (20% to 27%) are community-associated.[11,12]

CDI recurrences increase the burden to patients and the healthcare system, namely by prolonging hospitalization and decreasing quality of life (QoL).[13,14] Recurrent CDI is generally defined as an episode occurring between two to eight weeks after the onset of the previous episode,[15] and may result from a relapse of the same infection or re-infection with a new strain.[16,17] Approximately 10% to 30% of patients develop a recurrence after a primary CDI episode.[18,19]

Following the outbreaks associated with the emergence of RT027 in Europe, the European Centre for Disease Prevention and Control (ECDC) encouraged standardized surveillance of CDI at the country level.[20,21] In Portugal, National Health Service (NHS) laboratories must report all CDI episodes to the National Reference Laboratory (National Institute of Health Dr. Ricardo Jorge [INSA]) every trimester.[22] Fecal samples from a subset of CDI positive patients are also sent to INSA for the purpose of molecular characterization and antimicrobial susceptibility testing of *C. difficile* isolates.

The objective of this study was to provide a recent picture of CDI epidemiology and to characterize the clinical burden associated with this infection among hospitalized patients in Portugal. This characterization was complemented by a description of the molecular characteristics (polymerase chain reaction [PCR] ribotyping) and antimicrobial susceptibility of *C. difficile* isolates based on national surveillance for CDI.

MATERIAL AND METHODS

Study design

This was a retrospective, observational, multicenter study carried out in six public hospital centers (five tertiary and one secondary)[23] of the Portuguese NHS. The participating hospital centers served a total of 2 220 047 individuals in 2017.

All primary CDI episodes and related recurrences occurring between January and December 2017 were identified and recorded for patients meeting eligibility criteria. Recurrent episodes that developed between two to eight weeks after the last episode diagnosed in 2017 were also documented. Therefore, for each included patient, the observation period began at the onset of the first primary CDI episode diagnosed in 2017 and lasted up to eight weeks after the final episode identified that same year. Eligible patients included male and female inpatients aged ≥ 18 years at the time of the diagnosis of their first episode in 2017. Patients with an active chronic diarrheal illness or any condition leading to a frequent passage of loose stools (e.g., patients with ostomy) were excluded.

Data collected

Data were collected from patients' medical records and laboratory reports.

Sociodemographic data (age group and sex), comorbidities (diabetes, oncologic, cardiovascular, kidney, pulmonary, and inflammatory bowel disease), and recent history of parenteral nutrition, gastrointestinal surgery, nasogastric intubation, and enema at the time of the first primary CDI episode diagnosed in 2017 were recorded for each eligible patient.

CDI episodes were characterized based on the following variables: case origin (for primary episodes only), diagnostic tests performed, disease severity, and all cause in-hospital mortality. Case origin was defined as follows: healthcare-associated CDI (symptom onset [diarrhea] ≥ 3 days following hospital admission or within four weeks of discharge from any healthcare facility), community-associated CDI (symptom onset outside of the hospital within the previous 12 weeks or on the day of hospital admission or the following day), or unknown association (episodes in patients discharged from a healthcare facility four to 12 weeks before symptom onset). Disease severity (mild to moderate, severe, or severe with complications) was established based on the Society for Healthcare Epidemiology of America and on the Infectious Diseases Society of America guidelines.[24] It was also documented whether patients were on hemodialysis, immunosuppressed, receiving continuous steroid and/or proton pump inhibitor (PPI) treatment at the time of CDI diagnosis and if they had been exposed to antibiotics in the three months prior to each episode. Continuous steroid and/or PPI treatment were documented when patients were receiving these drugs both prior to and

after the CDI diagnosis was established.

In addition to the CDI cases diagnosed during the observation period, admissions resulting from CDI or during which these episodes developed were recorded. For each admission, we recorded the cause, the length of stay (LOS) in the hospital, intensive care unit (ICU), and general wards, and the post-discharge destination. The complete LOS and post-discharge destination were recorded, including for admissions where the patients were discharged after the observation period.

Case definition

The primary CDI episode was defined according to the ECDC surveillance protocol,[25] and met at least one of the following criteria: diarrheal stools or toxic megacolon and a positive laboratory assay for *C. difficile* toxin A and/or B in stools or a toxin-producing *C. difficile* organism detected in stool via culture or other means (e.g., positive PCR result); pseudomembranous colitis revealed by lower gastrointestinal endoscopy; colonic histopathology characteristic of CDI (with or without diarrhea) on a specimen obtained during endoscopy, colectomy, or autopsy. Only episodes for which no other CDI had been diagnosed in the previous eight weeks were classified as primary. CDI episodes were classified as recurrences when patients presented diarrheal stools again, and a positive laboratory test was obtained between two and eight weeks after the diagnosis of the previous episode.

National surveillance data

National surveillance data on CDI during 2017, including molecular characterization (PCR ribotyping) and antimicrobial susceptibility of *C. difficile* isolates, were provided by the National Reference Laboratory (INSA). RTs were determined by capillary gel electrophoresis-based PCR ribotyping, as described previously in the literature.[26,27] Susceptibility to metronidazole, moxifloxacin, and vancomycin was assessed by disk diffusion on *Brucella* agar with 5% sheep's blood, haemin and vitamin K1 (BBL; Becton Dickinson, San Diego, CA, USA), with anaerobic incubation at 37°C. Strains were classified as susceptible or resistant according to zone diameter breakpoints described by Erikstrup LT *et al*.[28]

Ethics

This study was approved by the Independent Ethics Committee (IECs) of each participating hospital center, all of which granted a waiver of informed consent. Approval was also obtained from INSA's IEC.

Statistical analysis

Analyses were conducted by patient, hospitalization and CDI episodes. Continuous variables were summarized by descriptive statistics, including mean, standard deviation, median, minimum and maximum values. Categorical variables were summarized by absolute and relative frequencies. Comparison of categorical variables between subgroups was performed with Chi-Square and Fisher's Exact tests.

The recurrence rate after a primary CDI episode was determined as the number of patients who developed a recurrent episode during the observation period divided by the number of patients with primary CDI (i.e., all included patients). A similar methodology was followed to calculate the rates of patients suffering from second, third, fourth, and fifth recurrences.

We determined the proportions of episodes occurring in patients with known risk factors for CDI development, overall and for primary and recurrent episodes separately. As we aimed to compare these proportions between primary and recurrent episodes, only variables collected for both types of episodes were considered for analysis – antibiotic use in the last three months, hemodialysis, immunosuppression, and continuous PPI treatment. Other variables that have been associated with increased CDI risk (e.g., older age and certain chronic conditions)[5,8,10] were not considered, as they were only documented for the first primary episode diagnosed in 2017.

Generalized estimating equation (GEE) models were carried out to assess which patient and episode characteristics were significantly associated with CDI severity and time to recurrence. Variables with a p value < 0.2 in univariate GEE models were included in the initial multivariate models. The optimized models included only variables showing a statistically significant association with CDI severity or time to recurrence. All statistical procedures were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA).

RESULTS

Three hundred and eighty-five patients with ≥ 1 primary CDI episode diagnosed in 2017 were included in the study. Six patients (1.6%) developed two primary episodes throughout this year. Patients had a mean of 1.2 CDI episodes (range: 1 to 6) during the observation period.

Patient characteristics

Patient characteristics at the time of the first primary episode recorded in 2017 are summarized in Table 1. Most patients were female (56.4%) and aged ≥ 70 years (73.2%). Approximately 90% of patients presented at least one comorbidity, with the most common being cardiovascular disease (80.5%). One hundred and five patients (27.3%) had three or more comorbidities. Recent history of nasogastric intubation was recorded for 17.7% of patients.

Patients with and without recurrent episodes were comparable in terms of age group (18 - 64 *vs* ≥ 65 years), sex, type and number of comorbidities, and recent history of parenteral nutrition, gastrointestinal surgery, nasogastric intubation, and enema.

Recurrence rates

Most patients (88.3%) did not have a recurrence following a primary episode (Fig. 1). Among the 45 patients who did, 10 (22.2%) had a second recurrence. Of these,

Table 1 – Patient characteristics at the time of the first primary CDI episode diagnosed in 2017, overall and for patients with and without recurrences

	Patients with recurrences (n = 45)	Patients without recurrences (n = 340)	Total (n = 385)	*p* value
Age group, n (%)				
18 - 29 years	0 (0.0%)	5 (1.5%)	5 (1.3%)	
30 - 39 years	0 (0.0%)	7 (2.1%)	7 (1.8%)	
40 - 49 years	1 (2.2%)	13 (3.8%)	14 (3.6%)	
50 - 59 years	4 (8.9%)	21 (6.2%)	25 (6.5%)	
60 - 64 years	4 (8.9%)	22 (6.5%)	26 (6.8%)	
65 - 69 years	2 (4.4%)	24 (7.1%)	26 (6.8%)	
≥ 70 years	34 (75.6%)	248 (72.9%)	282 (73.2%)	
18 - 64 years	9 (20.0%)	68 (20.0%)	77 (20.0%)	> 0.999
≥ 65 years	36 (80.0%)	272 (80.0%)	308 (80.0%)	
Sex, n (%)				
Female	25 (55.6%)	192 (56.5%)	217 (56.4%)	0.907
Male	20 (44.4%)	148 (43.5%)	168 (43.6%)	
Comorbidities of interest, n (%)				
≥ 1 Comorbidity of interest	41 (91.1%)	308 (90.6%)	349 (90.6%)	> 0.999
Diabetes	14 (34.1%)	101 (32.8%)	115 (33.0%)	0.862
Oncologic disease	11 (26.8%)	95 (30.8%)	106 (30.4%)	0.600
Cardiovascular disease	35 (85.4%)	246 (79.9%)	281 (80.5%)	0.404
Kidney disease	21 (51.2%)	122 (39.9%)	143 (41.2%)	0.166
Pulmonary disease	8 (19.5%)	50 (16.3%)	58 (16.7%)	0.603
Inflammatory bowel disease	0 (0.0%)	11 (3.6%)	11 (3.2%)	0.375
Number of comorbidities of interest, n (%)				
0	4 (8.9%)	32 (9.4%)	36 (9.4%)	0.940
1	13 (28.9%)	101 (29.7%)	114 (29.6%)	
2	14 (31.1%)	116 (34.1%)	130 (33.8%)	
≥ 3	14 (31.1%)	91 (26.8%)	105 (27.3%)	
Parenteral nutrition, n (%)				
Yes	2 (4.4%)	10 (2.9%)	12 (3.1%)	0.639
No	43 (95.6%)	330 (97.1%)	373 (96.9%)	
Gastrointestinal surgery, n (%)				
Yes	2 (4.4%)	42 (12.4%)	44 (11.4%)	0.117
No	43 (95.6%)	298 (87.6%)	341 (88.6%)	
Nasogastric tube, n (%)				
Yes	7 (15.6%)	61 (17.9%)	68 (17.7%)	0.693
No	38 (84.4%)	279 (82.1%)	317 (82.3%)	
Enema, n (%)				
Yes	0 (0.0%)	9 (2.7%)	9 (2.3%)	0.606
No	45 (100.0%)	330 (97.3%)	375 (97.7%)	

three (30.0%) suffered a third recurrent episode. Only one patient had more than three recurrences; this patient was diagnosed with five recurrent episodes throughout the observation period.

Hospitalization episodes

A total of 437 hospitalizations – resulting from CDI episodes or during which these developed – were recorded during the observation period. This represents 0.23% of all hospitalizations observed in the participating hospitals

throughout 2017 (190 891).

CDI was the cause of admission in 39.8% of the 437 hospitalizations recorded (Table 2). Median hospital LOS was 19 days. Patients were admitted to the ICU in 11.4% of hospitalizations, with a median LOS of nine days. Patients were discharged to their home in most hospitalizations (69.2%) for which the post-discharge destination was documented, with 27.1% being discharged to nursing homes or long-term care facilities.

CDI episodes

A total of 451 CDI episodes occurred during the observation period, the majority of which corresponded to primary episodes (86.7%). Most of the 60 recurrent episodes (75.0%) were first recurrences. There were 10 second recurrences, three third recurrences, and one fourth and fifth recurrences.

As for diagnostic tests, glutamate dehydrogenase (GDH) plus toxin test, arbitrated by nucleic acid amplification test (PCR), were performed in more than half of CDI episodes (n = 248; 55.7%). GDH plus toxin test were carried out in 176 episodes (39.6%), while the toxin test alone was used in 21 episodes (4.7%).

In terms of case origin, 49.1% of primary episodes were

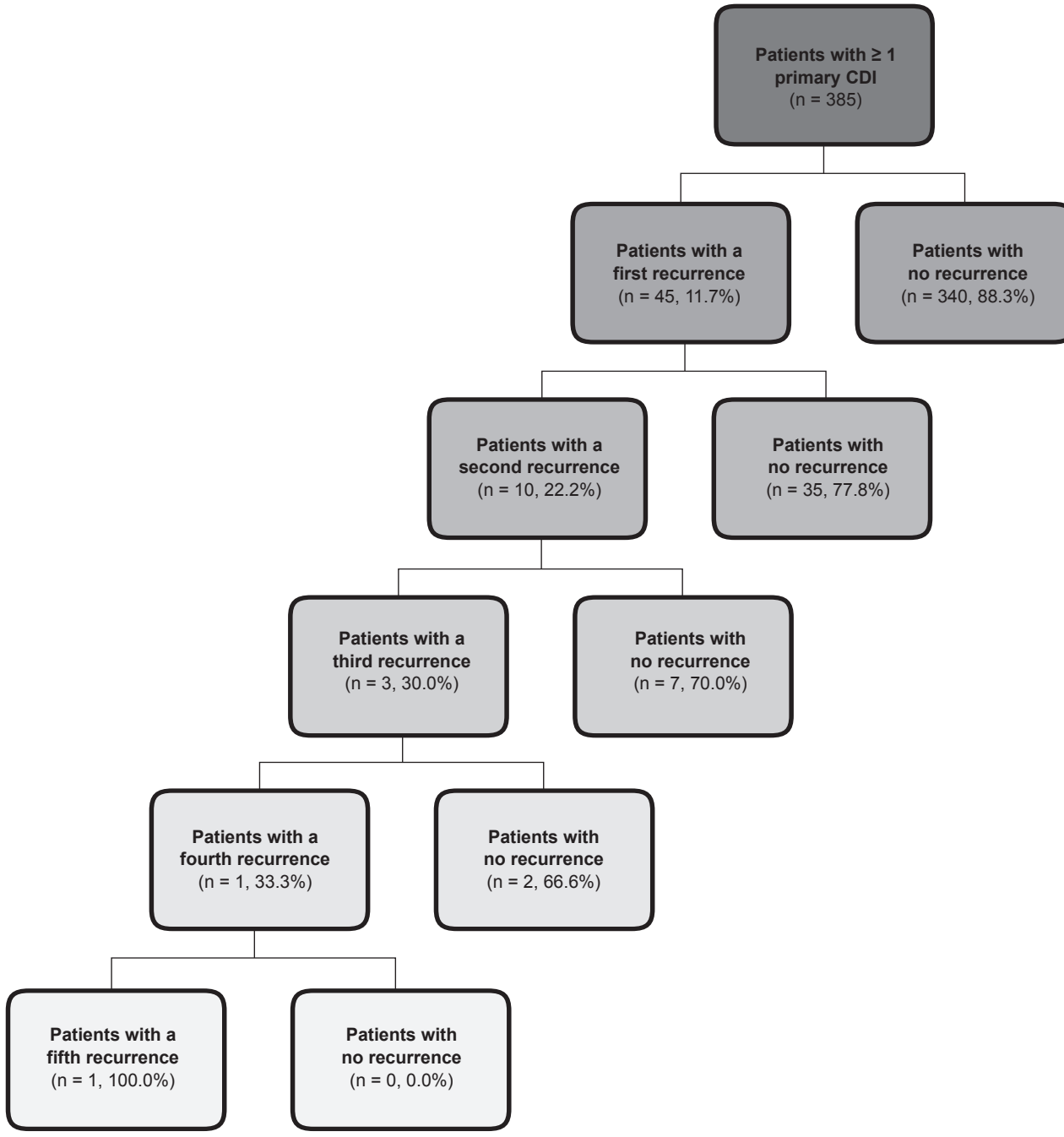

Figure 1 – Recurrence rates of inpatients with CDI episodes during the observation period

healthcare-associated CDI (Table 3). Most episodes had a mild to moderate severity (68.7%). All-cause in-hospital death was documented in 16.7% of episodes (75 out of 448 episodes with available data on mortality).

Primary and recurrent episodes were comparable in terms of infection severity and all-cause in-hospital death.

Risk factors for CDI development and use of concomitant medications

At least one known risk factor for CDI development was observed for most recorded episodes (94.9%), with the most common being recent antibiotic exposure (86.0%) – Table 4. The presence of risk factors (≥ 1) was similar between primary and recurrent episodes (94.1% *vs* 100.0%, respectively). However, recent antibiotic exposure was significantly more frequent among recurrent episodes (98.3% *vs* 84.1%; $p = 0.0030$).

Patients were on continuous steroid treatment in 9.2% of CDI episodes.

All cause in-hospital mortality

Seventy-five of the 385 patients (19.5%) died during the observation period, with a significantly higher all-cause in-hospital mortality being observed for patients aged ≥ 65 years when compared to those aged 18-64 years (22.4% *vs* 7.8%, respectively; $p = 0.0038$).

Risk factors for CDI severity and time to recurrence

Mild to moderate CDI episodes differed significantly from severe CDI episodes in terms of patients' age distribution – Appendix 1, Table 1 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/15890/Appendix_01.pdf). The proportion of older patients (≥ 65 years) was considerably higher for severe episodes (92.2% *vs* 74.5% for mild to moderate episodes; $p = 0.0001$). Based on the optimized GEE model, older patients have an increased odds of severe CDI (odds ratio = 1.44) compared to younger patients.

The mean time to recurrence among the 60 recurrent episodes recorded in this study was 29.4 days. The patients' age group significantly influenced the mean time to recurrence – Appendix 1, Table 2 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/15890/Appendix_01.pdf). Significantly lower mean times to recurrence were observed after CDI episodes occurring in patients aged 18 to 64 years (23.8 *vs* 30.8 days in those aged ≥ 65 years; $p = 0.0012$). Being older (≥ 65 years) increased the mean time to recurrence by 7.4 days compared to younger patients (18 – 64 years) according to the optimized GEE model.

National surveillance data

In 2017, INSA received a total of 206 non-duplicate stool samples from CDI positive patients aged ≥ 18 years old. Most patients were female (61.2%) and aged ≥ 65 years (72.8%).

C. difficile toxigenic strains – positive for toxins A and/or B – were recovered from the vast majority of stool samples (90.8%) – Appendix 1, Table 3 (Appendix 1: https://www.actamedicaportuguesa.com/revista/index.php/amp/article/view/15890/Appendix_01.pdf). Of these, 35 (18.7%) were also positive for the binary toxin (hypervirulent).

A great variety of RTs (over 50 different RTs) were observed among the *C. difficile* strains. The five most common PCR RTs among toxigenic strains (n = 187) were RT027 (8.6%), RT078/126 (8.6%), RT014 (8.0%), RT106

Table 2 – Hospitalization characteristics

	Total (n = 437)
Reason for hospital admission, n (%)	
C. difficile infection	174 (39.8%)
Other	263 (60.2%)
Hospital LOS (days)	
n	437
Mean	34.67
Median	19.00
Standard deviation	55.29
Minimum	1.00
Maximum	949.00*
ICU admission?, n (%)	
Yes	50 (11.4%)
No	387 (88.6%)
ICU LOS (days)	
n	50
Mean	13.10
Median	9.00
Standard deviation	13.37
Minimum	2.00
Maximum	79.00
General ward stay?, n (%)	
Yes	409 (93.6%)
No	28 (6.4%)
General ward LOS (days)	
n	409
Mean	33.74
Median	19.00
Standard deviation	55.97
Minimum	1.00
Maximum	949.00
Post-discharge destination, n (%)	
n	328
Home	227 (69.2%)
Transferred to another hospital	12 (3.7%)
Nursing home, long-term care facility	89 (27.1%)

LOS: length of stay; ICU: intensive care unit

*Excluding this outlier, the summary statistics for hospital LOS (days) are as follows: mean (standard deviation) = 32.58 (33.73); median = 19.00; minimum - maximum: 1.00 - 215.00.

Table 3 – CDI characteristics, overall and for primary and recurrent episodes

	Primary CDI episodes (n = 391)	Recurrent CDI episodes (n = 60)	Total (n = 451)	*p* value
CDI origin, n (%)				
Healthcare-associated CDI	192 (49.1%)	-	-	-
Community-associated CDI	174 (44.5%)	-	-	
Unknown association CDI	25 (6.4%)	-	-	
Infection severity, n (%)				
Mild to moderate	274 (70.1%)	36 (60.0%)	310 (68.7%)	0.1604
Severe	98 (25.1%)	22 (36.7%)	120 (26.6%)	
Severe with complications	19 (4.9%)	2 (3.3%)	21 (4.7%)	
All-cause in-hospital death, n (%)				
Yes	62 (16.0%)	13 (21.7%)	75 (16.7%)	0.2722
No	326 (84.0%)	47 (78.3%)	373 (83.3%)	
Missing values	3	0	3	

CDI: *Clostridioides difficile* infection

Table 4 – Risk factors for CDI development and use of concomitant medications, overall and for primary and recurrent episodes

	Primary CDI episodes (n = 391)	Recurrent CDI episodes (n = 60)	Total (n = 451)	*p* value
≥ 1 risk factor for CDI development, n (%)	368 (94.1%)	60 (100.0%)	428 (94.9%)	0.057
Antibiotic use in the last 3 months, n (%)*	327 (84.1%)	59 (98.3%)	386 (86.0%)	0.003
On hemodialysis, n (%)*	46 (11.8%)	5 (8.3%)	51 (11.3%)	0.431
Immunosuppressed, n (%)	117 (29.9%)	18 (30.0%)	135 (29.9%)	0.990
Continuous PPI treatment, n (%)	198 (50.6%)	30 (50.0%)	228 (50.6%)	0.926
Underlying reason for immunosuppression, n (%)				0.379
Comorbidities				
Cancer	75 (64.1%)	10 (55.6%)	85 (63.0%)	
HIV/AIDS	6 (5.1%)	2 (11.1%)	8 (5.9%)	
Transplantation procedures				
Bone marrow transplant/ hematopoietic stem cell transplant	3 (2.6%)	1 (5.6%)	4 (3.0%)	
Organ transplantation	16 (13.7%)	1 (5.6%)	17 (12.6%)	
Immunosuppressive therapy	17 (14.5%)	4 (22.2%)	21 (15.6%)	
Total	117 (100.0%)	18 (100.0%)	135 (100.0%)	
Concomitant medications, n (%)				
Continuous steroid treatment	35 (9.0%)	6 (10.0%)	41 (9.1%)	0.802

CDI: *Clostridioides difficile* infection; PPI: proton pump inhibitor; HIV: human immunodeficiency virus; AIDS: acquired immune deficiency syndrome
*: Percentages determined based on non-missing values

(7.5%), RT002 (5.9%), and RT020 (5.3%). Two of the most frequent genotypes were both hypervirulent strains (RT027 and RT078/126).

Regarding antibiotic susceptibility, all tested strains were susceptible to metronidazole and vancomycin. Thirty-nine (18.9%) strains were resistant to moxifloxacin, mostly associated with RT017, RT027, and RT078/126.

DISCUSSION

The present study aimed to evaluate the epidemiology of CDI and assess the clinical burden associated with this infection among hospitalized patients in Portugal.

Older patients (≥ 65 years) have been reported to be disproportionately affected by CDI, both in terms of incidence and mortality.[24,29] Our results support this tendency, as 80% of included patients were aged ≥ 65 years and 92% of recorded deaths occurred in this age group. Furthermore, most stool samples (72.8%) received by INSA in 2017 corresponded to patients aged ≥ 65 years. This study also found that older patients are at increased risk of severe CDI.

In the last decade, there has been an increase in CDI incidence among individuals without known risk factors, namely antibiotic-naïve patients, and those with minimal or no recent exposure to healthcare settings.[4] In this study, however, 94.9% of episodes occurred in patients

with known risk factors. Antibiotic exposure – the most important modifiable risk factor for CDI development[9] – was particularly common (observed in 86.0% of episodes). As indicated in previous research, these findings highlight the need for responsible use of antibiotics in order to reduce the healthcare burden associated with CDI.[1] The proportion of episodes for which patients presented risk factors may be even higher, as certain patient characteristics that have been associated with increased CDI risk (e.g., age ≥ 65 years and certain chronic conditions)[5,8,10] were only collected for the first primary episode diagnosed during 2017, and therefore were not considered in this analysis. Still, our data align with other studies showing that community-associated CDI has become responsible for a considerable proportion of all CDI cases,[5,30] as approximately 44% of primary episodes were community-associated.

CDI recurrences are associated with an increase in the overall disease burden to both patients and the healthcare system,[13,14] and it is estimated that 10% to 30% of CDI patients develop recurrences.[18,19] In the present study, the proportion of patients with recurrent disease (11.7%) was closer to the lower end of this range. The study methodology may, in part, be responsible for the lower rate observed. As the focus was placed on inpatients only, recurrent episodes occurring after hospital discharge were not captured if they did not lead to a new admission. Furthermore, episodes were not recorded when leading to admission in a non-study hospital.

Our study is consistent with previous research showing that the risk of recurrence increases with each consecutive recurrent episode.[18,19] In fact, the recurrence rate increased from 11.7% after a primary episode to approximately 20% and 30% following first and second recurrences, respectively.

Hypervirulent RT027 has been responsible for the increase in CDI incidence, severity, and mortality observed over recent decades.[3–5] Data from England shows that the prevalence of this strain is decreasing, having dropped from 55% to 21% within a three-year period (2007 to 2010). This reduction has been matched by a significant decrease in CDI-related mortality.[31] The comparison of the national surveillance data from 2017 with the study of CDI isolates conducted in 20 Portuguese hospitals between 2010-2015, which found a prevalence of 18.5% for RT027 among healthcare- and community-associated CDI isolates,[7] may suggest that the prevalence of this RT is also decreasing in Portugal.

INSA data support the existence of a considerable variety of *C. difficile* RTs in Portugal. The same conclusion had been made by Santos A *et al*, who identified a total of 96 distinct RTs among 498 *C. difficile* isolates recovered from different regions of mainland Portugal.[7] These findings are in line with the considerable diversity of *C. difficile* ribotypes that has been observed across Europe.[32]

This study had some limitations. First, the proportion of admissions at the participating hospital centers that were due to CDI or during which these episodes developed was determined based on the total number of admission episodes recorded at the hospital centers in 2017. However, we potentially excluded some admission episodes occurring in this year when related with recurrences of CDI episodes diagnosed in 2016. On the other hand, admissions occurring in 2018 were recorded when associated with recurrences of episodes diagnosed in 2017. Depending on whether the number of excluded admission episodes occurring in 2017 was higher or lower than that of included episodes recorded in 2018, the proportion of admissions due to CDI or during which these episodes developed may have been under- or overestimated. Second, we recorded recurrent episodes occurring up to eight weeks after the last CDI episode diagnosed in 2017. Patients could have experienced further recurrences after the end of the observation period which were not documented. This should be considered when interpreting results on recurrence rates.

CONCLUSION

In Portugal, hospitalized patients with CDI are mostly older patients (≥ 65 years) presenting with risk factors for the development of this infection, particularly recent antibiotic exposure. Mortality is disproportionately high among the older population. Community-associated CDI is common among hospitalized patients with this infection.

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AUTHORS CONTRIBUTION

CN, IL, ER, HI, FM, ER, FC, CS, SL, HM, NK, AS, MN: Conception, design, and development of the study; data acquisition; interpretation of results; writing and critical review of the manuscript.

LP, LP: Conception, design, and development of the study; interpretation of results; writing and critical review of the manuscript.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

COMPETING INTERESTS

Leonor Pássaro and Laura Paixão are employees of MSD Portugal. All remaining authors have no conflicts of interest to declare that are relevant to the content of this article.

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REFERENCES

1. Wiegand PN, Nathwani D, Wilcox MH, Stephens J, Shelbaya A, Haider S. Clinical and economic burden of Clostridium difficile infection in Europe: a systematic review of healthcare-facility-acquired infection. J Hosp Infect. 2012;81:1–14.
2. Peery AF, Dellon ES, Lund J, Crockett SD, McGowan CE, Bulsiewicz WJ, et al. Burden of gastrointestinal disease in the United States: 2012 Update. Gastroenterology. 2012;143:1179-87.e3.
3. Freeman J, Bauer MP, Baines SD, Corver J, Fawley WN, Goorhuis B, et al. The changing epidemiology of Clostridium difficile infections. Clin Microbiol Rev. 2010;23:529–49.
4. DePestel DD, Aronoff DM. Epidemiology of Clostridium difficile infection. J Pharm Pract. 2013;26:464–75.
5. Czepiel J, Dróżdż M, Pituch H, Kuijper EJ, Perucki W, Mielimonka A, et al. Clostridium difficile infection: review. Eur J Clin Microbiol Infect Dis. 2019;38:1211–21.
6. Ofosu A. Clostridium difficile infection: a review of current and emerging therapies. Ann Gastroenterol. 2016;29:147–54.
7. Santos A, Isidro J, Silva C, Boaventura L, Diogo J, Faustino A, et al. Molecular and epidemiologic study of Clostridium difficile reveals unusual heterogeneity in clinical strains circulating in different regions in Portugal. Clin Microbiol Infect. 2016;22:695–700.
8. Mada PK, Alam MU. Clostridium difficile. StatPearls, Treasure Island: StatPearls Publishing; 2020.
9. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, et al. Clinical Practice Guidelines for Clostridium difficile Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis. 2018;66:e1–48.
10. Heinlen L, Ballard JD. Clostridium difficile infection. Am J Med Sci. 2010;340:247–52.
11. Wilcox MH, Mooney L, Bendall R, Settle CD, Fawley WN. A case-control study of community-associated Clostridium difficile infection. J Antimicrob Chemother. 2008;62:388–96.
12. Kutty PK, Woods CW, Sena AC, Benoit SR, Naggie S, Frederick J, et al. Risk factors for and estimated incidence of community-associated Clostridium difficile infection, North Carolina, USA. Emerg Infect Dis. 2010;16:197–204.
13. Kelly CP. Can we identify patients at high risk of recurrent Clostridium difficile infection? Clin Microbiol Infect Dis. 2012;18:S21–7.
14. Johnson S. Recurrent Clostridium difficile infection: a review of risk factors, treatments, and outcomes. J Infect. 2009;58:403–10.
15. Debast SB, Bauer MP, Kuijper EJ, European Society of Clinical Microbiology and Infectious Diseases. European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for Clostridium difficile infection. Clin Microbiol Infect. 2014;20:S1–26.
16. Singh T, Bedi P, Bumrah K, Singh J, Rai M, Seelam S. Updates in treatment of recurrent Clostridium difficile infection. J Clin Med Res. 2019;11:465–71.
17. Song JH, Kim YS. Recurrent Clostridium difficile infection: risk factors, treatment, and prevention. Gut Liver. 2019;13:16–24.
18. Fekety R, McFarland LV, Surawicz CM, Greenberg RN, Elmer GW, Mulligan ME. Recurrent Clostridium difficile diarrhea: characteristics of and risk factors for patients enrolled in a prospective, randomized, double-blinded trial. Clin Infect Dis. 1997;24:324–33.
19. McFarland LV, Surawicz CM, Rubin M, Fekety R, Elmer GW, Greenberg RN. Recurrent Clostridium difficile disease: epidemiology and clinical characteristics. Infect Control Hosp Epidemiol. 1999;20:43–50.
20. Dorp SM van, Kinross P, Gastmeier P, Behnke M, Kola A, Delmée M, et al. Standardised surveillance of Clostridium difficile infection in European acute care hospitals: a pilot study, 2013. Eurosurveillance. 2016;21:30293.
21. Kuijper EJ, Coignard B, Tüll P. Emergence of Clostridium difficile-associated disease in North America and Europe. Clin Microbiol Infect. 2006;12:2–18.
22. Direção-Geral da Saúde. Norma 004/2013 de 08 de Agosto de 2013, atualizada a 13 de Novembro de 2015, intitulada "Vigilância Epidemiológica das Resistências aos Antimicrobianos". 2015. [cited 2020 Nov 10]. Available from: https://www.dgs.pt/directrizes-da-dgs/normas-e-circulares-normativas/norma-n-0042013-de-21022013-jpg.aspx.
23. European Centre For Disease Prevention and Control. Surveillance Report - Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals. 2013. [cited 2020 Nov 9]. Available from: https://www.ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/healthcare-associated-infections-antimicrobial-use-PPS.pdf.
24. Cohen SH, Gerding DN, Johnson S, Kelly CP, Loo VG, McDonald LC, et al. Clinical Practice Guidelines for Clostridium difficile Infection in Adults: 2010 Update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). Infect Control Hosp Epidemiol. 2010;31:431–55.
25. European Centre For Disease Prevention and Control. European Surveillance of Clostridioides (Clostridium) difficile infections. 2019. [cited 2020 Nov 9]. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/clostridium-difficile-infections-EU-surveillance-protocol-vers2.4.pdf.
26. Indra A, Huhulescu S, Schneeweis M, Hasenberger P, Kernbichler S, Fiedler A, et al. Characterization of Clostridium difficile isolates using capillary gel electrophoresis-based PCR ribotyping. J Med Microbiol. 2008;57:1377–82.
27. Fawley WN, Knetsch CW, MacCannell DR, Harmanus C, Du T, Mulvey MR, et al. Development and validation of an internationally-standardized, high-resolution capillary gel-based electrophoresis PCR-ribotyping protocol for Clostridium difficile. PloS One. 2015;10:e0118150.
28. Erikstrup LT, Danielsen TK, Hall V, Olsen KE, Kristensen B, Kahlmeter G, et al. Antimicrobial susceptibility testing of Clostridium difficile using EUCAST epidemiological cut-off values and disk diffusion correlates. Clin Microbiol Infect. 2012;18:E266-72.
29. Pépin J, Valiquette L, Cossette B. Mortality attributable to nosocomial Clostridium difficile-associated disease during an epidemic caused by a hypervirulent strain in Quebec. CMAJ. 2005;173:1037–42.
30. Khanna S, Pardi DS, Aronson SL, Kammer PP, Orenstein R, St Sauver JL, et al. The epidemiology of community-acquired Clostridium difficile infection: a population-based study. Am J Gastroenterol. 2012;107:89–95.
31. Wilcox MH, Shetty N, Fawley WN, Shemko M, Coen P, Birtles A, et al. Changing epidemiology of Clostridium difficile infection following the introduction of a national ribotyping-based surveillance scheme in England. Clin Infect Dis. 2012;55:1056–63.
32. Davies KA, Ashwin H, Longshaw CM, Burns DA, Davis GL, Wilcox MH, et al. Diversity of Clostridium difficile PCR ribotypes in Europe: results from the European, multicentre, prospective, biannual, point-prevalence study of Clostridium difficile infection in hospitalised patients with diarrhoea (EUCLID), 2012 and 2013. Euro Surveill Bull. 2016;21.