Intensive & Critical Care Nursing 53 (2019) 73–78

Contents lists available at ScienceDirect

Intensive & Critical Care Nursing

journal homepage: www.elsevier.com/iccn

Research article

Characteristics, risk factors and outcomes of *Clostridium difficile* infections in Greek Intensive Care Units

Dimitrios K. Matthaiou [a,1], Dimitra Delga [a,1], Maria Daganou [b], Antonia Koutsoukou [b], Niki Karabela [c], Konstantinos E. Mandragos [c], Eleni Kalogeropoulou [d], George Dimopoulos [a,*]

[a] Department of Critical Care Medicine, "ATTIKON" University Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece
[b] Department of Critical Care, "SOTIRIA" Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece
[c] Department of Critical Care, "Korgialenio Benakio" Red Cross General Hospital, Athens, Greece
[d] Department of Microbiology, "ATTIKON" University Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece

ARTICLE INFO

Article history:
Accepted 19 March 2019

Keywords:
C. difficile
ICU
Critically-ill
Mortality

ABSTRACT

Background: *Clostridium difficile* is one of the major causes of diarrhoea among critically ill patients and its prevalence increases exponentially in relation to the use of antibiotics and medical devices. We sought to investigate the incidence of *C. difficile* infection in Greek units, and identify potential risk factors related to *C. difficile* infection.
Methods: A prospective multicenter cohort analysis of critically ill patients (3 ICUs from 1/1/2014 to 31/12/2014).
Results: Among 970(100%) patients, 95(9.79%) with diarrhoea, were included. Their demographic, comorbidity and clinical characteristics were recorded on admission to the unit. The known predisposing factors for the infection were recorded and the diagnostic tests to confirm *C. difficile* were conducted, based on the current guidelines. The incidence of *C. difficile* infection was 1.3% (n = 13). All-cause mortality in patients with diarrhoea, *C. difficile* infection and attributable mortality in patients with *C. difficile* infection was 28%, 38.5% and 30.8% respectively. Sequential Organ Failure Assessment (SOFA) scores on admission were significantly lower and prior *C. difficile* infection was more common in patients with current *C. difficile* infection. Regarding other potential risk factors, no difference was found between groups. No factor was independently associated with *C. difficile* infection.
Conclusions: *C. difficile* infection is low in Greek intensive care units, but remains a serious problem among the critically-ill. Mortality was similar to reports from other countries. No factor was independently associated with *C. difficile* infection.

Implications for clinical practice

- The prevalence of *Clostridium difficile* infection (CDI) in critically-ill patients is variable among ICUs.
- Risk factors for CDI are hard to define and may include non-adherence to infection control, hand-hygiene and good clinical practice protocols.
- Prior history of CDI is more common in patients who will demonstrate a new episode of CDI.

Introduction

Clostridium difficile, an anaerobic Gram-positive sporogonous and toxigenic bacillus, was first described in 1935 but was identified as a cause for antibiotic-associated pseudomembranous colitis

* Corresponding author at: Department of Critical Care Medicine, ATTIKON University Hospital, 1 Rimini Str., 124 62 Haidari, Greece.
E-mail address: gdimop@med.uoa.gr (G. Dimopoulos).
[1] Equal contribution.

https://doi.org/10.1016/j.iccn.2019.03.008
0964-3397/

in 1978 (Bartlett, 2002). Carrier incidence among healthy adults ranges between 3 and 5% and its colonisation increases significantly after the age of 65 (Samore, 1993). Outside hospital settings *C. difficile* colonisation ranges between 10 and 20% with most patients remaining asymptomatic. Among the elderly hospitalised patients, its asymptomatic presence reaches 14%, and 30% among those under antibiotic treatment (Samore, 1993; Labbe et al., 2008; Modena et al., 2005; Voth and Ballard, 2005). In hospital settings, both symptomatic and asymptomatic patents are considered to be the main source of environmental contamination, since *C. difficile* is present in their stool and it is communicated through the stool-oral route (Modena et al., 2005). Apart from this, its spores can remain active for a long time on hospital surfaces and its strains seem to be resistant to alcohol-based disinfectants. In cases when inappropriate disinfectants are used, its incidence increases (Cohen et al., 2010).

C. difficile infection (CDI) accounts for 30% of all hospital diarrhoea and as high as 50–70% of patients receiving antibiotic treatment (Samore, 1993; Oldfield, 2004; Riley, 2004; Garey et al., 2006). CDI epidemiology has changed after 2000, since 2005 there has been an observed increase in its frequency and severity, the presence of the new virulent strain, development in medical interventions and changes in hospitalised patients' characteristics (Kuijper et al., 2006). Furthermore, because of the increased use of cephalosporins, CDI is the most common cause of antibiotic-associated diarrhoea (Khanna and Pardi, 2012; Marshall, 2004).

Due to the fact that CDI is observed in patients with already serious comorbidities, diarrhoea causes even more weakness and as a result CDI is associated with significant mortality, reaching almost 20% within the first month since diagnosis and 27% three months after it. However, mortality rates fluctuate and in some studies are estimated to be as high as 85%, due to multiple risk factors. Determining CDI incidence in ICU is difficult because many patients are admitted either due to the infection or after a colectomy.

Mortality due to CDI, especially in the ICU setting, is not significantly higher compared to overall hospital mortality. However, CDI may considerably increase ICU length of stay. This in turn contributes to increased overall mortality related to the prolonged length of ICU stay and the exposure to other risk factors for mortality (Kenneally et al., 2007; Dodek et al., 2013). It should also be noted, that there are reports of decreasing CDI incidence over the past decade (Bouza et al., 2015). Factors contributing to this decrease include infection control and antibiotic stewardship measures, as well as increased vigilance for the possibility of CDI.

The aim of this prospective study was to estimate CDI incidence in ICUs in Greece and identify special indicators or factors associated with it among the critically-ill.

Methods

The study was conducted in three ICUs from 1/1/2014 to 31/12/2014. All patients who were hospitalised for more than 72 hours and developed diarrhoea at some point during their hospitalisation were included in the study. Informed consent for participation in the study was taken either from the patients themselves of from first-degree relatives. The study protocol was approved by the scientific boards of each of the participating hospitals. The Ethics approval number for the participating hospitals are 1. 914/11-12-13 for Attikon University Hospital 2. 4719/17-2-14 for Sotiria Hospital 3. 1096/16-1-14 for Korgialeneio Hospital.

Inclusion and exclusion criteria

A patient was deemed eligible to participate in the study if they were hospitalised in the ICU for more than 72 hours for any reason and developed diarrhoea defined by at least three watery defecations per day (scale 5 to 7 according to the Bristol Stool Chart) (Lewis and Heaton, 1997). A patient was deemed ineligible to participate in the study if they denied consent, were younger than 18 years old, had already developed diarrhoea upon admission in the ICU, had hepatic failure (scale 3 to 4 according to Child-Pugh score) or had inflammatory bowel disease.

Data collection

Relevant data were collected in a case report form including demographics, somatometric data (Labbe et al., 2008), reason for admission (Modena et al., 2005), disease severity scores (APACHE II, SOFA and Charlson Comorbidity Index (CCI) (Charlson et al., 1987), comorbidities and potential risk factors for CDI. Charlson Comorbidity Index was calculated, during the first 72 hours of hospitalisation in the ICU.

Definitions

CDI was clinically defined according to the 2010 guidelines of the Society for Healthcare Epidemiology of America and the Infectious Diseases Society of America (Cohen et al., 2010) if a patient manifested at least three watery defecations, which tested positive for *C. difficile* and any of the following clinical signs: leukocytosis >15,000/mm^3, dehydration/hypotension, shock, ileus and megacolon. If there was a positive culture for *C. difficile*, then the isolate was tested for Clostridium toxins A and B to confirm CDI. Laboratory diagnosis was performed by simultaneous detection of glutamate dehydrogenase (GDH) and toxin A and B by a stool specimen, followed by a toxigenic culture (TC). If GDH and toxins A and B were detected then CDI was confirmed. If CDI was confirmed, new stool specimens were obtained on days three and five after the onset of symptoms. If a patient did not yield positive laboratory findings for CDI after an episode of diarrhoea, he could be assessed for a subsequent episode of diarrhoea after a minimum of seven days (Goldenberg and French, 2011; Pancholi et al., 2012). The severity of CDI was defined according to the UK Health Protection Agency (UK Department of Health, 2008) as: Bartlett (2002) mild, if there was a normal leucocyte count and up to three watery defecations per day belonging to a scale from 5 to 7 according to the Bristol Stool Chart; Samore (1993) moderate, if the leucocyte count was up to 15000/mm^3 and 3 to 5 watery defecations; Labbe et al. (2008) severe, if the leucocyte count was higher than 15,000/mm^3, creatinine increased more than 50% of the baseline, the patient temperature was more than 38.5 °C and there were clinical or imaging signs of severe colitis and Modena et al. (2005) life-threatening, if there were imaging signs of severe colitis, hypotension, incomplete or complete ileus and toxic megacolon.

Attributable mortality to CDI was defined as death due to the infection with *Clostridium difficile*, if it ensued because of severe sepsis or septic shock or organ dysfunction; it was deemed to be a sequela of the infection with the pathogen in regard and if it was not best attributed to concurrent or subsequent infection with another pathogen or other comorbidities.

Outcomes

We sought to assess the incidence of CDI in patients hospitalised in the ICU, the incidence of diarrhoea in the ICU, as well as the proportion of patients whose diarrheic episodes were due to *C. difficile*. Other outcomes included the infection and patient outcome, which were assessed on the 28th day after the initial episode. Furthermore, additional analysis to detect potential risk factors for CDI in ICU patients were performed.

Statistical analysis

Patients with diarrhoea were divided based on whether they had CDI or not. A univariate analysis was performed between the two groups. The chi-square or the Fisher's Exact tests were used, when appropriate in the comparisons regarding categorical variables, whereas the *t*-test or the Mann-Whitney *U* Test were used in the comparisons regarding continuous variables, depending on the normality of distribution. A p value of <0.05 was regarded as indicative of statistical significance. Factors found to be significantly associated with the acquisition of CDI were introduced in a logistic regression model to identify independent predictors. Goodness of fit was assessed with Hosmer-Lemeshow Test. The analyses were performed with the SPSS software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp).

Results

Overall, 970 (100%) patients were included in the study, 95 (9.79%) presented with diarrhoea and 13 (1.34%) had a positive stool culture for *C. difficile*. All variables for ICU patients with and without CDI, as well as outcomes are summarised in Table 1. Thirty-eight (40%) patients were male. The mean age was 65.2 ± 14.9 years and the mean weight was 81.5 ± 15.9 kg. Regarding disease severity, the APACHE II, SOFA and CCI were 19.5 ± 8.7, 6.5 ± 3.1 and 4.5 ± 2.2, respectively. Patients without CDI had a higher SOFA score upon admission (p = 0.034).

The most common comorbities were diabetes mellitus (n = 35, 36.8%), followed by Chronic Obstructive Pulmonary Disease (n = 25, 26.3%). Other comorbidities included heart failure (n = 16, 16.8%), chronic renal disease (n = 11, 11.6%), stroke (either ischaemic or haemorrhagic) (n = 13, 13.7%), cancer (n = 6, 6.3%), and haematological malignancy (n = 1, 1.1%). There was no difference in comorbidities between patients with CDI versus those without CDI. Twenty-eight patients (29.5%) had a previous hospitalisation and thirteen patients (13.7%) had a prolonged one. Three patients (3.2%) were receiving renal replacement therapy, one (1.2%) had a gastrostomy and one (1.2%) had a colostomy. Two patients (2.1%) had a previous CDI. There was no difference regarding prior medical history between the groups, with the exception of prior CDI, which was more common in patients with current CDI (p = 0.017).

The most common reason for admission to the ICU admission was pneumonia (n = 30, 31.6%). Other reasons included ARDS (n = 26, 27.4%), sepsis (n = 17, 17.9%), surgery (n = 14, 14.7%), cardiovascular disease (n = 9, 9.5%) and gastrointestinal bleeding (n = 1, 1.2%). Twenty-one patients (22.1%) were admitted for various other reasons. There was no difference regarding reasons for admission in the ICU between the groups. Regarding previous concomitant treatment, the most common one was proton-pump inhibitors, which was received by sixty-six patients (69.5%). Other drugs included low molecular weight heparin (n = 34, 35.8%), glucocorticoids (n = 30, 31.6%), laxatives (n = 12, 12.6%), H2-blockers (n = 11, 11.6%), heparin (n = 8, 8.4%), acenocumarol (n = 4, 4.2%), chemotherapy (n = 3, 3.2%), gastrokinetics (n = 3, 3.2%), non-steroidal anti-inflammatory drugs (n = 2, 2.1%) and anti-retrovirals (n = 1, 1.1%). There was no difference regarding previous concomitant treatment between the groups.

The most common previous antibiotic treatment was piperacillin/tazobactam which was received by twenty-seven patients (28.4%), followed by carbapenems (n = 20, 21.1%). Other antibiotics included third generation cephalosporins (n = 19, 20%), vancomycin (n = 19, 20%), b-lactam/b-lactam inhibitor (n = 11, 11.6%), linezolid (n = 10, 10.5%), colistin (n = 10, 10.5%), moxifloxacin (n = 8, 8.4%), macrolides (n = 8, 8.4%), second generation cephalosporins (n = 7, 7.4%), aminoglycosides (n = 6, 6.3%), clindamycin (n = 5, 5.3%), ciprofloxacin (n = 3, 3.2%), metronidazole (n = 2, 2.1%) and tigecycline (n = 2, 2.1%). There was no difference regarding previous antibiotic treatment between the groups. During ICU hospitalisation, the most common antibiotic treatment were carbapenems which were administered to thirty-one patients (32.6%), followed by third generation cephalosporins (n = 23, 24.2%). Other antibiotics included vancomycin (n = 20, 21.1%), colistin (n = 16, 16.8%), linezolid (n = 14, 14.7%), moxifloxacin (n = 11, 11.6%), b-lactam/b-lactam inhibitor (n = 11, 11.6%), piperacillin/tazobactam (n = 10, 10.5%), metronidazole (n = 10, 10.5%), clindamycin (n = 6, 6.3%), macrolides (n = 5, 5.3%), ciprofloxacin (n = 4, 4.2%), aminoglycosides (n = 3, 3.2%), tigecycline (n = 3, 3.2%) and second generation cephalosporins (n = 1, 1.1%). There was no difference regarding antibiotic treatment during ICU hospitalisation between the groups. There was no difference also between the groups in leukocytosis, creatinine increase >50% of baseline, hypotension, and shock. Furthermore, there was no difference between the groups in signs suggestive of CDI such as ileus and megacolon.

Regarding the outcomes, twenty-eight patients (29.5%) died within 28 days after admission in the ICU. There was no difference in mortality between the groups. The death of four patients (30.8%) with CDI was attributable to the infection. Eight patients (61.5%) were considered cured from the CDI or improved within the same period. A logistic regression including all potential risk factors for CDI was performed, namely SOFA score upon admission and history of prior CDI infection. Hosmer and Lemeshow test suggested the model was a good fit to the data as p = 0.84. No factor was independently associated with CDI.

Discussion

The main finding of our study was that the incidence of CDI was 1.3%, the mortality was 38.5% and the attributable mortality was 30.8%. Although SOFA upon admission and history of prior CDI infection were significantly different between patients with or without CDI, no independent association was found with CDI.

Contrary to relevant bibliography, no differences were found in patients with and without CDI in any of the other parameters including antibiotic consumption, comorbidities, proton-pump inhibitors, demographics and length of stay. Relevant bibliography reports several potential risk factors for CDI, either initial or recurrent, including renal disease, antibiotic use, strain type, use of PPIs, age etc. (Abou Chakra et al., 2014; Deshpande et al., 2015; Zilberberg et al., 2014; Gutierrez-Pizarraya et al., 2018). In our cohort, although SOFA score upon admission and prior CDI were found to be significantly different between the groups, none of them was independently associated with CDI. It should be noted that SOFA score upon admission was significantly lower in patients with CDI in our cohort. Given that age, co-morbidities and other parameters, either directly or indirectly contributing in the calculation of SOFA score, are considered to be risk factors for CDI, this finding does not seem to have an obvious explanation. A possible reason for this difference could be a prolonged length of stay and administration of antibiotics for less severely ill patients, as more severely ill patients have increased mortality. However, no difference in these parameters was found between groups. Although a sensitivity analysis based on length of stay and duration of antibiotics combined with severity of illness could further shed light on the issue, such an analysis was not performed because the patient samples were too small to allow a meaningful conclusion.

The identified incidence of CDI (1.3%) is rather low, given the increased antibiotic consumption in Greece. This finding is comparable with the incidence reported in other countries and settings (Gutierrez-Pizarraya et al., 2018; Segar et al., 2017; Skoutelis et al.,

Table 1
Comparison between ICU patients with diarrhoea and with and without *C. difficile* infection.

	C. difficile (−) culture n = 82 (%)	*C. difficile* (+) culture n = 13 (%)	*p*
Male	32 (39)	6 (46.2)	0.63
Age	65.2 ± 14.3	65.3 ± 19.1	0.59
Weight	80.9 ± 14.5	85.2 ± 22.9	0.86
APACHE II	19.8 ± 8.5	17.2 ± 9.8	0.53
SOFA	6.8 ± 3.2	4.8 ± 1.7	0.034
CCI	4.5 ± 2.1	4.5 ± 2.3	0.75
Reason of ICU admission			
Pneumonia	28 (34.1)	2 (15.4)	0.22
Sepsis	12 (14.6)	5 (38.5)	0.053
ARDS	24 (29.3)	2 (15.4)	0.50
Gastrointestinal bleeding	1 (1.2)	0 (0)	1.00
Cardiovascular disease	9 (11)	0 (0)	0.35
Surgery	11 (13.4)	3 (23.1)	0.40
Other	18 (22)	3 (23.1)	1.00
Comorbidities			
Diabetes mellitus	30 (36.6)	5 (38.5)	1.00
Heart failure	13 (15.9)	3 (23.1)	0.45
Chronic renal disease	10 (12.2)	1 (7.7)	1.00
COPD	23 (28)	2 (15.4)	0.50
Cancer	6 (7.3)	0 (0)	0.59
Haematological malignancy	1 (1.2)	0 (0)	1.00
Ischaemic stroke	4 (4.9)	1 (7.7)	0.53
Acute haemorrhagic stroke	6 (7.3)	2 (15.4)	0.30
Prior medical history			
Prior hospitalisation	23 (28)	5 (38.5)	0.52
Prolonged hospitalisation	11 (13.4)	2 (15.4)	1.00
Renal replacement therapy	3 (3.7)	0 (0)	1.00
Gastrostomy	1 (1.2)	0 (0)	1.00
Colostomy	1 (1.2)	0 (0)	1.00
Prior CDI	0 (0)	2 (15.4)	0.017
Concomitant treatment			
Proton-pump inhibitors	58 (70.7)	8 (61.5)	0.53
H_2-blockers	10 (12.2)	1 (7.7)	1.00
Acenocumarol	3 (3.7)	1 (7.7)	0.45
LMWH	31 (37.8)	3 (23.1)	0.37
Heparin	5 (6.1)	3 (23.1)	0.075
Glucocorticoids	28 (34.1)	2 (15.4)	0.22
Chemotherapy	2 (2.4)	1 (7.7)	0.36
NSAIDs	2 (2.4)	0 (0)	1.00
Laxatives	10 (12.2)	2 (15.4)	0.67
Gastrokinetics	2 (2.4)	1 (7.7)	0.36
Anti-retrovirals	1 (1.2)	0 (0)	1.00
Prior antibiotic treatment			
b-lactam/b-lactam inhibitors	11 (13.4)	0 (0)	0.35
2nd generation cephalosporins	5 (6.1)	2 (15.4)	0.24
3rd generation cephalosporins	15 (18.3)	4 (30.8)	0.25
Piperacillin/tazobactam	25 (30.5)	2 (15.4)	0.34
Ciprofloxacin	3 (3.7)	0 (0)	1.00
Moxifloxacin	7 (8.5)	1 (7.7)	1.00
Aminoglycosides	5 (6.1)	1 (7.7)	1.00
Carbapenems	16 (19.5)	4 (30.8)	0.46
Vancomycin	15 (18.3)	4 (30.8)	0.29
Clindamycin	3 (3.7)	2 (15.4)	0.14
Macrolides	8 (9.8)	0(0)	0.59
Metronidazole	2 (2.4)	0 (0)	1.00
Linezolid	10 (12.2)	0 (0)	0.35
Tigecycline	1 (1.2)	1 (7.7)	0.26
Colistin	9 (11)	1 (7.7)	1.00
Antibiotic treatment			
b-lactam/b-lactam inhibitors	10 (12.2)	1 (7.7)	1.00
2nd generation cephalosporins	1 (1.2)	0 (0)	1.00
3rd generation cephalosporins	20 (24.4)	3 (23.1)	1.00
Piperacillin/tazobactam	10 (12.2)	0 (0)	0.35
Ciprofloxacin	4 (4.9)	0 (0)	1.00
Moxifloxacin	10 (12.2)	1 (7.7)	1.00
Aminoglycosides	2 (2.4)	1 (7.7)	0.36
Carbapenems	27 (32.9)	4 (30.8)	1.00
Vancomycin	16 (19.5)	4 (30.8)	0.46
Clindamycin	4 (4.9)	2 (15.4)	0.19
Macrolides	5 (6.1)	0 (0)	1.00
Metronidazole	10 (12.3)	0 (0)	0.35

Table 1 (*continued*)

	C. difficile (−) culture n = 82 (%)	*C. difficile* (+) culture n = 13 (%)	*p*
Linezolid	14 (17.1)	0 (0)	0.20
Tigecycline	2 (2.4)	1 (7.7)	0.36
Colistin	12 (14.6)	4 (30.8)	0.22
CDI criteria			
Leukocytosis	46 (56.1)	8 (61.5)	0.71
Creatinine increase > 50% of baseline	29 (35.4)	8 (61.5)	0.07
Hypotension	12 (14.6)	1 (7.7)	0.69
Shock	2 (2.4)	0 (0)	1.00
Ileus	3 (3.7)	0 (0)	1.00
Megacolon	0 (0)	1 (7.7)	0.14
Outcomes			
Mortality	23 (28)	5 (38.5)	0.52
Attributable mortality to CDI		4 (30.8)	

APACHE II = Acute Physiology and Chronic Health Evaluation II, SOFA = Sequential Organ Failure Assessment, CCI = Charlson Comorbidity Index, ARDS = Acute Respiratory Distress Syndrome, COPD = Chronic Obstructive Pulmonary Disease, LMWH = Low Molecular Weight Heparin, NSAIDs = Non Steroidal Anti-Inflammatory Drugs.

2017; Yoon et al., 2014; Tirlapur et al., 2016). It should be noted that our cohort consisted of a mixed population. This fact may potentially underestimate the incidence compared to populations with high morbidity, such as patients with malignancies (Apostolopoulou et al., 2011). However, our population are representative of the patient mix that is generally admitted in the Greek ICUs.

It should be noted that similar incidence was also found in other studies coming from Greece. A recent study by Samonis et al. (2016) reports a 6% prevalence of positive culture of *C. difficile* in hospitalised patients with diarrhoea and 4% prevalence of positive culture and positive toxin by enzyme immunoassay. Kachrimanidou et al. (2017) report an incidence of 25 CDIs per 10,000 hospital admissions, while of 33 CDI cases, 72.7% were hospital-acquired. It is interesting that the hypervirulent PCR ribotype 027 was not found, whereas ribotypes 017 and 126 predominated.

Specifically for the ICU setting, our results are similar with a recent meta-analysis Karanika et al. (2016) which found that the prevalence of CDI among ICU patients was 2%, and among diarrheic ICU patients the prevalence was 11%. The overall hospital mortality among ICU patients with CDI was 32%, compared with 24% among those without CDI presenting a statistically significant difference in mortality risk. The authors reached a similar conclusion, highlighting the need for additional prevention in this setting.

On the other hand, the low prevalence of CDI within our cohort may need per se the inclusion of a larger number of individuals to show any potential risk factors. However, given that there is no reason to support that the incidence would rise if the cohort was larger, even if potential risk factors would be found their clinical impact would be marginal. It seems that there may be factors that have not been quantified up to now in the available bibliography that may have a role in the prevalence of CDI.

Overall mortality in both of our groups was 29.5%, while overall mortality and attributable mortality in patients with CDI was 38.5% and 30.8%, respectively. Mortality in our group is similar with reports from other countries (Bouza et al., 2015; Karanika et al., 2016; Alvarez-Lerma et al. 2014). However, attributable mortality to CDI and the impact of CDI on length of stay are outcomes which are not so extensively researched (Kenneally et al., 2007; Yoon et al., 2014). It has been suggested that CDI is associated with increased risk for mortality up to more than 50%, while it may prolong the length of stay, although in the study by Dodek et al. no association of CDI with ICU or hospital mortality was found (Dodek et al., 2013; Karanika et al., 2016; Reacher et al., 2016). The timing of CDI treatment seems to be important, as the early treatment of ICU-acquired CDI is not independently associated with an increased mortality and impacts marginally the ICU length of stay (Zahar et al., 2012). In our cohort, attributable mortality and overall mortality in the *C. difficile* positive group are almost the same, most probably because of the small number of non-survivors in this group. However, the overall mortality and attributable mortality to CDI in the cohort were 29.5% and 4.2%, respectively.

During the past decade, there has been an increase in a specific type of *C. difficile*, which is hypervirulent, namely ribotype 027 complex. It has been associated with higher mortality and severity of disease (Awad et al., 2014; Valiente et al., 2014). Although toxins A and B are major virulence factors of the pathogen, their detection in the stool by enzyme immunoassay does not predict the severity of CDI or mortality (Rao et al., 2015). Thus, PCR ribotyping may be essential to detect the existence of the hypervirulent strain and predict worse outcomes. It should be noted that ribotyping was not performed in our cohort. Given the relatively high attributable mortality, the presence of the hypervirulent strain cannot be excluded.

Given that infection control is difficult in the ICUs, especially if one considers the relative faecal abundance and the colonisation pressure that are common in the ICU setting, it is important to control the reservoir, improve hand hygiene and control the environment. Irrespective to the variable prevalence of CDI among ICUs, it should be stressed out that strict hand-washing may be one of the key factors for infection control. Hand disinfection with alcoholic solutions is ineffective. Instead, thorough hand-washing using soap and soap should be performed. The promotion of hand hygiene among health care workers in the ICUs may greatly contribute to the control or even reduction in this setting (Zahar and Blot, 2018; De Wandel, 2017; Battistella et al., 2017).

Limitations

There are some limitations that should be considered. First, the design of our study may have missed potential factors that would increase the risk of acquisition of CDI that could have an impact on the outcomes. Also, one may argue that the case mix is rather heterogeneous to allow meaningful conclusions, as well as the small number of participating ICUs. It should be noted that the structure of the health system in Greece does not support the diversity in case mix, as the majority of Greek ICUs are mixed ICUs with similar case mix. The ICUs included in our study are among the largest in Greece. Regarding the clinical practices, we acknowledge the existence of potential differences. However, they most probably lie on the implementation of infection control measures, which is the reason we suggest it as the main reason of the low incidence of CDI in our cohort. Furthermore, one may argue

that the low prevalence of CDI was unexpected given the usually reported high exposure rate to antibiotics in the Greek ICU population. Given that no risk factors have been identified, it seems that there may be factors that have not been quantified up to now in the available bibliography that may have a role in the prevalence of CDI. We think that the implementation of infection control measures and bundles have a crucial role, although this could not be quantified with the applied methodology in our study. Lastly, this study may depict cross-sectional data that do not essentially uniformly represent clinical practice.

Conclusion

In conclusion, CDI was found to have alow incidence in critically ill patients in Greek ICUs. Mortality was similar to reports from other countries. Although SOFA score upon admission was lower in patients with CDI and history of prior CDI infection was higher compared to patients without CDI, no factor was independently associated with CDI.

Conflict of interest

The authors have no conflict of interest to declare.

Ethical statement

The study protocol was approved by the scientific boards of each of the participating hospitals. Informed consent for participation in the study was taken either from the patients themselves of from first-degree relatives.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.iccn.2019.03.008.

References

Abou Chakra, C.N., Pepin, J., Sirard, S., Valiquette, L., 2014. Risk factors for recurrence, complications and mortality in *Clostridium difficile* infection: a systematic review. PLoS One 9, (6) e98400.

Alvarez-Lerma, F., Palomar, M., Villasboa, A., Amador, J., Almirall, J., Posada, M.P., et al., 2014. Epidemiological study of *Clostridium difficile* infection in critical patients admitted to the Intensive Care Unit. Med. Intensiva 38 (9), 558–566.

Apostolopoulou, E., Raftopoulos, V., Terzis, K., Elefsiniotis, I., 2011. Infection Probability Score: a predictor of Clostridium difficile-associated disease onset in patients with haematological malignancy. Eur. J. Oncol. Nurs. 15 (5), 404–409.

Awad, M.M., Johanesen, P.A., Carter, G.P., Rose, E., Lyras, D., 2014. *Clostridium difficile* virulence factors: insights into an anaerobic spore-forming pathogen. Gut Microbes 5 (5), 579–593.

Bartlett, J.G., 2002. Clinical practice. Antibiotic-associated diarrhea. N. Engl. J. Med. 346 (5), 334–339.

Battistella, G., Berto, G., Bazzo, S., 2017. Developing professional habits of hand hygiene in intensive care settings: An action-research intervention. Intensive Crit. Care Nurs. 38, 53–59.

Bouza, E., Rodriguez-Creixems, M., Alcala, L., Marin, M., De Egea, V., Braojos, F., et al., 2015. Is *Clostridium difficile* infection an increasingly common severe disease in adult intensive care units? A 10-year experience. J. Crit. Care 30 (3), 543–549.

Charlson, M.E., Pompei, P., Ales, K.L., MacKenzie, C.R., 1987. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J. Chronic Dis. 40 (5), 373–383.

Cohen, S.H., Gerding, D.N., Johnson, S., Kelly, C.P., Loo, V.G., McDonald, L.C., et al., 2010. 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. 31 (5), 431–455.

De Wandel, D., 2017. Key factors for hand hygiene promotion in intensive care units. Intensive Crit. Care Nurs. 42, 3–4.

Deshpande, A., Pasupuleti, V., Thota, P., Pant, C., Rolston, D.D., Hernandez, A.V., et al., 2015. Risk factors for recurrent *Clostridium difficile* infection: a systematic review and meta-analysis. Infect. Control Hosp. Epidemiol. 36 (4), 452–460.

Dodek, P.M., Norena, M., Ayas, N.T., Romney, M., Wong, H., 2013. Length of stay and mortality due to Clostridium difficile infection acquired in the intensive care unit. J. Crit. Care 28 (4), 335–340.

Garey, K.W., Graham, G., Gerard, L., Dao, T., Jiang, Z.D., Price, M., et al., 2006. Prevalence of diarrhea at a university hospital and association with modifiable risk factors. Ann. Pharmacother. 40 (6), 1030–1034.

Goldenberg, S.D., French, G.L., 2011. Diagnostic testing for *Clostridium difficile*: a comprehensive survey of laboratories in England. J. Hosp. Infect. 79 (1), 4–7.

Gutierrez-Pizarraya, A., Martin-Villen, L., Alcala-Hernandez, L., Marin Arriaza, M., Balandin-Moreno, B., Aragon-Gonzalez, C., et al., 2018. Epidemiology and risk factors for Clostridium difficile infection in critically ill patients in Spain: The PROCRID study. Enferm. Infect. Microbiol. Clin. 36 (4), 218–221.

Kachrimanidou, M., Tsachouridou, O., Ziogas, I.A., Christaki, E., Protonotariou, E., Metallidis, S., et al., 2017. Clostridium difficile infections in a university hospital in Greece are mainly associated with PCR ribotypes 017 and 126. J. Med. Microbiol. 66, 1774–1781.

Karanika, S., Paudel, S., Zervou, F.N., Grigoras, C., Zacharioudakis, I.M., Mylonakis, E., 2016. Prevalence and clinical outcomes of clostridium difficile infection in the intensive care unit a systematic review and meta-analysis. Open Forum Infect. Dis. 3 (1), ofv186.

Kenneally, C., Rosini, J.M., Skrupky, L.P., Doherty, J.A., Hollands, J.M., Martinez, E., et al., 2007. Analysis of 30-day mortality for clostridium difficile-associated disease in the ICU setting. Chest 132 (2), 418–424.

Khanna, S., Pardi, D.S., 2012. *Clostridium difficile* infection: new insights into management. Mayo Clin. Proc. 87 (11), 1106–1117.

Kuijper, E.J., Coignard, B., Tull, P., Difficile ESGfC, States EUM, European Centre for Disease P, et al., 2006. Emergence of *Clostridium difficile*-associated disease in North America and Europe. Clin. Microbiol. Infect. 12 (Suppl. 6), 2–18.

Labbe, A.C., Poirier, L., Maccannell, D., Louie, T., Savoie, M., Beliveau, C., et al., 2008. *Clostridium difficile* infections in a Canadian tertiary care hospital before and during a regional epidemic associated with the BI/NAP1/027 strain. Antimicrob. Agents Chemother. 52 (9), 3180–3187.

Lewis, S.J., Heaton, K.W., 1997. Stool form scale as a useful guide to intestinal transit time. Scand. J. Gastroenterol. 32 (9), 920–924.

Marshall, J.C., 2004. Intra-abdominal infections. Microbes Infect. 6 (11), 1015–1025.

Modena, S., Bearelly, D., Swartz, K., Friedenberg, F.K., 2005. *Clostridium difficile* among hospitalized patients receiving antibiotics: a case-control study. Infect. Control Hosp. Epidemiol. 26 (8), 685–690.

Oldfield 3rd., E.C., 2004. *Clostridium difficile*-associated diarrhea: risk factors, diagnostic methods, and treatment. Rev. Gastroenterol. Disord. 4 (4), 186–195.

Pancholi, P., Kelly, C., Raczkowski, M., Balada-Llasat, J.M., 2012. Detection of toxigenic *Clostridium difficile*: comparison of the cell culture neutralization, Xpert C. difficile, Xpert C. difficile/Epi, and Illumigene C. difficile assays. J. Clin. Microbiol. 50 (4), 1331–1335.

Rao, K., Micic, D., Natarajan, M., Winters, S., Kiel, M.J., Walk, S.T., et al., 2015. *Clostridium difficile* ribotype 027: relationship to age, detectability of toxins A or B in stool with rapid testing, severe infection, and mortality. Clin. Infect. Dis. 61 (2), 233–241.

Reacher, M., Verlander, N.Q., Roddick, I., Trundle, C., Brown, N., Farrington, M., et al., 2016. Excess mortality attributable to *Clostridium difficile* and risk factors for infection in an historic cohort of hospitalised patients followed up in the united kingdom death register. PLoS One 11, (3) e0149983.

Riley, T.V., 2004. Nosocomial diarrhoea due to *Clostridium difficile*. Curr. Opin. Infect Dis. 17 (4), 323–327.

Samonis, G., Vardakas, K.Z., Tansarli, G.S., Dimopoulou, D., Papadimitriou, G., Kofteridis, D.P., et al., 2016. Clostridium difficile in Crete, Greece: epidemiology, microbiology and clinical disease. Epidemiol. Infect. 144, 161–170.

Samore, M.H., 1993. Epidemiology of nosocomial *Clostridium difficile* infection. Compr. Ther. 19 (4), 151–156.

Segar, L., Easow, J.M., Srirangaraj, S., Hanifah, M., Joseph, N.M., Seetha, K.S., 2017. Prevalence of *Clostridium difficile* infection among the patients attending a tertiary care teaching hospital. Ind. J. Path Microb. 60 (2), 221–225.

Skoutelis, A., Pefanis, A., Tsiodras, S., Sipsas, N.V., Lelekis, M., Lazanas, M.C., et al., 2017. Point-prevalence survey of healthcare facility-onset healthcare-associated *Clostridium difficile* infection in Greek hospitals outside the intensive care unit: the C. Define study. PLoS One 12, (8) e0182799.

Tirlapur, N., Puthucheary, Z.A., Cooper, J.A., Sanders, J., Coen, P.G., Moonesinghe, S.R., et al., 2016. Diarrhea in the critically ill is common, associated with poor outcome, and rarely due to *Clostridium difficile*. Sci. Rep. 6, 24691.

UK Department of Health, 2008. *Clostridium difficile* Infection: How to Deal with the Problem. Department of Health, London, UK.

Valiente, E., Cairns, M.D., Wren, B.W., 2014. The *Clostridium difficile* PCR ribotype 027 lineage: a pathogen on the move. Clin. Microbiol. Infect. 20 (5), 396–404.

Voth, D.E., Ballard, J.D., 2005. *Clostridium difficile* toxins: mechanism of action and role in disease. Clin. Microbiol. Rev. 18 (2), 247–263.

Yoon, Y.K., Kim, M.J., Sohn, J.W., Kim, H.S., Choi, Y.J., Kim, J.S., et al., 2014. Predictors of mortality attributable to *Clostridium difficile* infection in patients with underlying malignancy. Support. Care Cancer 22 (8), 2039–2048.

Zahar, J.R., Blot, S., 2018. Dilemmas in infection control in the intensive care unit. Intensive Crit. Care Nurs. 46, 1–3.

Zahar, J.R., Schwebel, C., Adrie, C., Garrouste-Orgeas, M., Francais, A., Vesin, A., et al., 2012. Outcome of ICU patients with *Clostridium difficile* infection. Crit. Care 16 (6), R215.

Zilberberg, M.D., Reske, K., Olsen, M., Yan, Y., Dubberke, E.R., 2014. Risk factors for recurrent *Clostridium difficile* infection (CDI) hospitalization among hospitalized patients with an initial CDI episode: a retrospective cohort study. BMC Infect. Dis. 14, 306.