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Clostridium difficile in Crete, Greece: epidemiology, microbiology and clinical disease

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SUMMARY

We studied the epidemiology and microbiology of *Clostridium difficile* and the characteristics of patients with *C. difficile* infection (CDI) in Crete in three groups of hospitalized patients with diarrhoea: group 1 [positive culture and positive toxin by enzyme immunoassay (EIA)]; group 2 (positive culture, negative toxin); group 3 (negative culture, negative toxin). Patients in group 1 were designated as those with definitive CDI (20 patients for whom data was available) and matched with cases in group 2 (40 patients) and group 3 (40 patients). *C. difficile* grew from 6% (263/4379) of stool specimens; 14·4% of these had positive EIA, of which 3% were resistant to metronidazole. Three isolates had decreased vancomycin susceptibility. Patients in groups 1 and 2 received more antibiotics ($P = 0{\cdot}03$) and had more infectious episodes ($P = 0{\cdot}03$) than patients in group 3 prior to diarrhoea. Antibiotic administration for *C. difficile* did not differ between groups 1 and 2. Mortality was similar in all three groups (10%, 12·5% and 5%, $P = 0{\cdot}49$). CDI frequency was low in the University Hospital of Crete and isolates were susceptible to metronidazole and vancomycin.

Key words: *C. difficile*-associated diarrhoea, *C. difficile* mortality, *C. difficile* resistance, *C. difficile* susceptibility, *C. difficile* toxin.

INTRODUCTION

Clostridium difficile infection (CDI) has been increasingly reported as a threat to public health during the last decade. In the United States in 2013, CDC estimated that *C. difficile* accounted for or prolonged the duration of hospitalization of 250000 infections annually, 14000 deaths, and at least 1 billion dollars in excess medical costs [1]. CDI severity ranges from self-limiting diarrhoea to life-threatening pseudomembranous colitis [2, 3]. Prior uses of antibiotics, hospitalization or residency in healthcare facilities, and ageing have been identified among the risk factors for the development of CDI [3–7]. Despite the fact that the prevalence of CDI has been increasing worldwide [8, 9], this clinical entity is still underestimated by clinicians and frequently under-diagnosed [10].

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Diagnosis of CDI is also troublesome. Toxigenic cultures (the gold standard) are rarely performed in daily practice, because they are time consuming [10, 11]. The sensitivity of the two-step method, which utilizes detection of glutamate dehydrogenase in stool and positive toxin A or B enzyme immunoassay (EIA, 75–95%) is lower than that of polymerase chain reaction (PCR); which in turn is more expensive and currently cannot be widely used [11, 12]. As a result, most cases in clinical practice are defined by a positive-toxin EIA in a patient with history and symptoms compatible with CDI [11]. This definition has also been used in several clinical studies [10, 13–16]. In addition, a delay in transportation or preparation for toxin detection may increase the possibility of false-negative results [17]. Hence, diarrhoea episodes that are true CDI cases may be misclassified as non-CDI. On the other hand, it is known that non-toxigenic *C. difficile* strains may prevent colonization from toxigenic *C. difficile* strains and CDI [18].

In this article we sought to evaluate the epidemiology and microbiology of *C. difficile* and study the characteristics of patients with CDI in the island of Crete. In addition, we sought to study whether there are differences between patients with diarrhoea and positive culture and toxin EIA and those with positive culture but negative toxin EIA, including history, prescribed treatment and all-cause in-hospital mortality.

METHODS

Study design, setting and patient population

A retrospective study was performed using data from the University Hospital of Heraklion, Crete, Greece, which is a 700-bed tertiary-care general hospital and operates as a reference centre for the island's 620 000 inhabitants. All *C. difficile* strains isolated from diarrhoeal stool samples between 2004 and 2010 were identified using the electronic records of the Department of Microbiology. The available patient charts were used for extraction of clinical data. Approval was given by the ethics committee of the University Hospital of Heraklion, Crete. Patient's records/information were anonymized.

At the University Hospital of Heraklion in Crete, Greece, all patients with clinical suspicion of CDI are tested. However, it is not mandatory to test all patients with compatible symptoms and history or all diarrhoeal specimens for CDI. Reporting to national or regional agencies is also not mandatory for CDI. A culture for identification of *C. difficile* is performed and toxin production is tested via EIA in all diarrhoeal stool samples submitted for possible CDI. Stool samples sent to the microbiology department for testing of possible CDI were classified as having positive or negative culture for *C. difficile* and toxin A and B immunoassay test. The samples were classified in three groups. Group 1 consisted of those with positive culture and positive toxin EIA (C+T+). Group 2 included those with positive culture and negative toxin (C+T–). Group 3 consisted of those with negative culture and negative toxin (C–T–).

Any patient with a positive culture for *C. difficile* and toxin (C+T+), regardless of history of prior CDI (i.e. both primary episodes and recurrences), was eligible for inclusion in the study. Only one positive stool sample per patient episode was included in the study. If more than one episodes of CDI occurred in the same patient, any subsequent episode could be included in the analysis if it occurred at least 1 month after the resolution of the previous episode.

The available medical records of patients with C+T+ stool samples (group 1) were searched for data extraction. Data regarding demographic characteristics and patients' history (including data from the index hospitalization period prior to the development of diarrhoea) were collected via pre-specified forms and tabulated. Interventions during hospitalization and antibiotic treatment were also recorded. Data regarding other bacteria or fungi isolated from the same clinical specimen, different specimens at the same time or variable specimens at different time points prior to or after the index diarrhoeal episode were also recorded.

A matched case-case-control study was done. Matching was done for gender, year of isolation and department of admission at the time of isolation. Year of isolation and department of admission were selected in order to reduce discrepancies in the diagnostic approach and treatment for possible CDI. The patients with C+T+ stool samples were matched to patients with C+T– stool samples in a 1:2 ratio. Matching was done consecutively for gender, year of isolation and department of admission. If more than two patients could be matched to one C+/T+ case, the patients were selected randomly using random numbers. A third group consisting of patients with diarrhoea who tested negative for culture and toxin (C–T–) for *C. difficile* (group 3) was created using the previously described methodology in a 1:2 ratio (C+T+:C–T–), and served as the control group.

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Microbiological assays

All diarrhoeal stool specimens sent to the microbiology department for toxin A and B identification were also cultured for *C. difficile*. The presence of *C. difficile* toxins A and B in stools was determined using Immunocard Toxins A+B (Meridian Bioscience, USA). Stool samples were cultured on *C. difficile* selective agar (bioMérieux, France) and incubated for 48 h at 37 °C. Isolates were presumptively identified by characteristic colony morphology, smell, fluorescence under UV light and appearance on Gram stain. Species identification was performed using a commercial biochemical identification system (Rapid ID 32A, bioMérieux). Toxigenic cultures were not performed.

Isolates were tested against ampicillin, cefoxitin, cefotaxime, cefepime, chloramphenicol, tetracycline, erythromycin, clindamycin, rifampicin, metronidazole and vancomycin, using E-test strips (AB Biodisk, Sweden). These antibiotics are included in the panel used for determining the susceptibility of the anaerobes, according to the protocols of our laboratory. A suspension of *C. difficile* equivalent to 1 McFarland turbidity standard was spread on *Brucella* agar supplemented with haemin and vitamin K1 (BD Diagnostic Systems, USA), and incubated anaerobically at 37 °C. The minimum inhibitory concentration (MIC) was read after 24 h (except for clindamycin which was read after 48 h). Results were interpreted according to 2011 Clinical and Laboratory Standards Institute (CLSI) criteria [19]. The European Committee for Antimicrobial Susceptibility Testing (EUCAST 2012) criteria for susceptibility to vancomycin were used (susceptible isolates with MIC $\leqslant 2\,\mu$g/ml) [20], since CLSI did not provide breakpoints for vancomycin. Reference strains (*Bacteroides fragilis* ATCC 25 285, *B. thetaiotaomicron* ATCC 29741 and *C. difficile* 700 057) were included as controls to monitor the antimicrobial susceptibility testing.

Definitions and outcomes

CDI diagnosis was deemed definitive in a patient with diarrhoea (>2 loose bowel movements per day), with or without other signs and symptoms compatible with CDI, and a C+T+ stool sample. A patient with diarrhoea and C+T– stool specimen was considered colonized by *C. difficile*. The severity of CDI was determined according to published criteria [21]. In brief, CDI was considered severe when one or more of the following was present: fever (>38·5 °C) or rigor, signs of peritonitis or ileus, marked leucocytosis (>15 000/μl) or left shift (>20% band cells), pseudomembranous colitis, megacolon, findings of bowel wall thickening or pericolonic fat stranding in computed tomography, ascites, shock, acute respiratory distress syndrome, acute renal failure, multi-organ failure, and lactic acidosis not otherwise explained. Prior infection was considered any infection in the previous 3 months from the development of diarrhoea. Concurrent bacterial infection was defined as any infection developing 5 days prior to or after the development of diarrhoea. An episode of diarrhoea was considered hospital-acquired if it developed at least 2 days after admission.

Data analysis and statistical methods

The χ^2 test or Fisher's exact tests were used, as appropriate, for comparisons regarding categorical variables, whereas the *t* test was used in comparisons regarding continuous variables. The Shapiro–Wilk test was used for assessment of variable distribution. For non-normally distributed continuous variables, the Mann–Whitney or the Wilcoxon signed-rank test was used. A *P* value of <0·05 was regarded as indicative of statistical significance. When data were not available, patients were excluded from the analysis. The comparisons were performed with SPSS software v. 17.0 (SPPS Inc., USA).

RESULTS

Epidemiology

During the study period 293 941 patients were admitted in the hospital. Table 1 shows that the number of admitted patients varied in the studied years, as did the number of stool specimens. A total of 4379 patients with diarrhoea provided a stool specimen for suspected CDI during the 7-year period (2004–2010); the number of tests performed for possible CDI increased gradually with time ($P = 0{\cdot}048$). In 263 (6%) tests *C. difficile* was grown. The frequency of *C. difficile* isolation in diarrhoeal stool specimens varied significantly each year (from 2·8% to 8·2%, $P < 0{\cdot}001$, Fig. 1). The highest frequency was seen in the years 2005, 2008 and 2009 and the lowest in 2004 and 2010. Of specimens with positive culture for *C. difficile*, the toxin EIA was positive in 38 (14·4%). The frequency of CDI in culture-positive patients varied each year from 6·5% up to 22·6%; the low frequency observed each year did not allow further meaningful statistical analyses. The frequency of C+T+ stool samples tested for possible CDI in all samples during the study was 0·87%.

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Table 1. *Frequency of colonization and infection with* C. difficile *during a 7-year period in the University Hospital of Heraklion*

Study year	No. of hospital admissions	All cultured specimens (diarrhoea)	Specimens cultured for *C. difficile* (%)*	Positive cultures for *C. difficile* (%)†	Toxin A+B tests positive (%)‡
2004	40717	1767	428 (19·5)	12 (2·8%)	1 (8·3%)
2005	43136	1959	569 (22·5)	45 (7·9)%	9 (20%)
2006	41907	2383	606 (20·3)	31 (5·1%)	7 (22·6%)
2007	41505	2291	695 (23·3)	39 (5·6%)	6 (15·4%)
2008	39867	2471	753 (23·4)	62 (8·2%)	4 (6·5%)
2009	42831	2032	672 (24·9)	52 (7·7%)	7 (13·4%)
2010	43978	2102	656 (23·9)	22 (3·4%)	4 (18·2%)
2004–2010	**293941**	**15005**	**4379 (22·6)**	**263 (6%)**	**38 (14·4%)**

* Percentage refers to specimens cultured for *C. difficile* in all stool specimens.
† Percentage refers to positive cultures in cultures performed for isolation of *C. difficile.*
‡ Percentage refers to positive enzyme immunoassay in positive cultures for *C. difficile.*

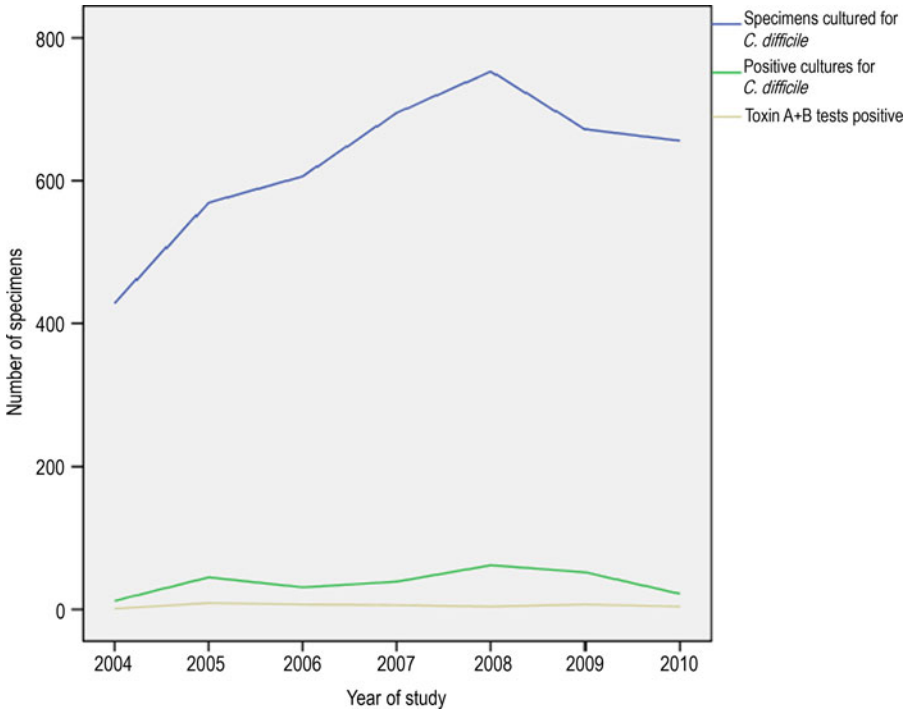

Fig. 1. Number of cultures performed for possible *Clostridium difficile* infection, number of positive cultures, and number of positive cultures with a simultaneous positive enzyme immunoassay through the study years.

Antimicrobial susceptibility

All isolates were resistant to cephalosporins. In addition, 86% of the isolates were resistant to clindamycin, 41% to erythromycin, 19% to rifampicin, 18% to tetracycline, and 5% to chloramphenicol. Intermediate susceptibility was found in 22%, 14%, and 8% of the isolates to erythromycin, tetracycline and chloramphenicol, respectively. The lower resistance was found to ampicillin (2%) and metronidazole (3%). None of the isolates was resistant to vancomycin, but three isolates had an MIC of 2 μg/ml.

Patient characteristics

The medical records of 20/38 patients with C+T+ stool samples, for whom data was available, were retrieved and comprised group 1. These patients were matched with 40 patients each in groups 2 and 3, according to

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Table 2. *Characteristics and outcomes of patients included in the study*

Variable	Group 1 culture(+)/toxin(+) ($N = 20$), *n* (%)	Group 2 culture(+)/toxin(–) ($N = 40$), *n* (%)	Group 3 culture(–)/toxin(–) ($N = 40$), *n* (%)	*P* value ‖	Group 3 *vs.* group 1 OR (95% CI)	Group 3 *vs.* group 2 OR (95% CI)	Group 2 *vs.* group 1 OR (95% CI)
Demographics							
Males	11 (55)	22 (55)	22 (55)	–			
Age, years, median (range)	68 (32–96)	73 (19–89)	73 (18–91)	0·96			
Comorbidity							
Diabetes mellitus	4/20 (20)	6/40 (15)	2/40 (5)	0·18			
Cardiac disease	8/20 (40)	23/40 (57·5)	16/40 (40)	0·23			
Respiratory disease	5/20 (25)	6/40 (15)	5/40 (12·5)	0·45			
Renal disease	3/20 (15)	7/40 (17·5)	2/40 (5)	0·21			
ARF on admission	1/20 (5)	6/40 (15)	8/40 (20)	0·31			
Inflammatory bowel disease	2/20 (10)	7/40 (17·5)	8/40 (20)	0·62			
CNS disease	6/20 (30)	7/40 (17·5)	9/40 (22·5)	0·54			
Cancer	3/20 (15)	8/40 (20)	9/40 (22·5)	0·79			
Immunosuppression	9/20 (45)	26/40 (65)	21/40 (52·5)	0·29			
Prior hospitalization	14/20 (70)	21/40 (52·5)	17/40 (42·5)	0·13			
Prior infection(s)	7/20 (35)	15/40 (37·5)	5/40 (12·5)	**0·03**	**0·27 (0·07–0·99)**	**0·24 (0·08–0·74)**	1·11 (0·36–3·42)
Prior surgery	6/20 (30)	9/40 (22·5)	7/40 (17·5)	0·54			
Radiation therapy	1/20 (5)	4/40 (10)	4/40 (10)	0·78			
Central venous catheter	3/20 (15)	7/40 (17·5)	4/40 (10)	0·62			
Nasogastric tube	1/20 (5)	11/40 (27·5)	4/40 (10)	**0·03**	2·11 (0·22–20·25)	**0·29 (0·08–1·02)**	7·21 (0·86–60·48)
Parenteral nutrition	7/20 (35)	6/40 (15)	2/40 (5)	**0·009**	**0·10 (0·02–0·53)**	0·30 (0·06–1·58)	0·33 (0·09–1·16)
CVVHF	2/20 (10)	3/40 (7·5)	0/40 (0)	0·16			
Intubation	3/20 (15)	9/40 (22·5)	4/40 (10)	0·31			
Medications							
PPIs	7/20 (35)	25/40 (62·5)	28/40 (70)	**0·03**	**4·33 (1·39–13·56)**	1·4 (0·55–3·55)	**3·09 (1·01–9·46)**
H_2-blockers	2/20 (10)	6/40 (15)	3/40 (7·5)	0·56			
ACEIs/ARBs	5/20 (25)	22/40 (55)	11/40 (27·5)	**0·02**	1·13 (0·33–3·88)	**0·31 (0·12–0·79)**	**3·67 (1·12–12·03)**
Statins	7/20 (35)	13/40 (32·5)	6/40 (15)	0·12			
Steroids	6/20 (30)	17/40 (42·5)	17/40 (42·5)	0·59			
Interferon	1/20 (5)	1/40 (2·5)	0/40 (0)	0·41			
Anti-TNF	0/20 (0)	3/40 (7·5)	1/40 (2·5)	0·31			
Anti-neoplasmatic drugs	2/20 (10)	10/40 (25)	13/40 (32·5)	0·17			
Prior antibiotic treatment (3 mos.)	13/19 (65)	22/35 (55)	13/40 (32·5)	**0·03**	**0·26 (0·08–0·80)**	**0·39 (0·16–0·98)**	0·66 (0·21–2·00)
Probiotics	3/20 (15)	3/40 (7·5)	0/40 (0)	0·06			
Concurrent bacterial infection(s)	3/20 (15)*	13/40 (32·5)†	22/40 (55)‡	**0·007**	**6·92 (1·75–27·43)**	**2·54 (1·02–6·30)**	2·73 (0·68–11·00)
Fever	2/20 (10%)	22/40 (55%)	15/40 (37·5)	**0·003**	**5·40 (1·10–26·61)**	0·49 (0·20–1·20)	**11·0 (2·25–53·84)**

Table 2 (*cont.*)

Variable	Group 1 culture(+)/toxin(+) ($N = 20$), *n* (%)	Group 2 culture(+)/toxin(–) ($N = 40$), *n* (%)	Group 3 culture(–)/toxin(–) ($N = 40$), *n* (%)	*P* value ‖	Group 3 *vs.* group 1 OR (95% CI)	Group 3 *vs.* group 2 OR (95% CI)	Group 2 *vs.* group 1 OR (95% CI)
Hospital-acquired diarrhoea	11/20 (55)	14/40 (35)	–§	0·14			
Duration of hospitalization, days, median (range)	9·5 (1–160)	8·5 (2–60)	9 (2–62)	0·69			

ACEIs, Angiotensin converting enzyme inhibitors; ARBs, angiotensin receptor blockers; ARF, acute renal failure; CI, confidence interval; CNS, central nervous system; CVVHF, continuous veno-venous haemofiltration; OR, odds ratio; PPIs, proton pump inhibitors; TNF, tumour necrosis factor.

* Two intra-abdominal infections, one respiratory tract infection

† Three respiratory tract infections, seven urinary tract infections, two intra-abdominal infections, one candidaemia.

‡ Eight respiratory tract infections, two urinary tract infections, three intra-abdominal infections (one gastroenteritis), nine other sites of unknown origin.

§ Data regarding the date of culture was not collected.

‖ Denotes that difference exists or not between groups, not for specific comparisons.

gender, department of admission and year. The characteristics of the included patients are presented in Table 2.

No significant differences were observed between patients in the three groups with regard to age, comorbidity and prior hospitalization. Patients in groups 1 and 2 received more antibiotics (65% and 55% *vs.* 32·5%, $P = 0·03$) and had more documented infections compared to patients in group 3 (35% and 37·5% *vs.* 12·5%, $P = 0·03$) prior to the development of diarrhoea. Patients in group 1 received more commonly parenteral nutrition (35% *vs.* 15% and 5%, $P = 0·009$), while this difference between groups 2 and 3 did not reach statistical significance ($P = 0·08$). Patients in group 1 also received fewer proton pump inhibitors (PPIs) before the development of diarrhoea (35% *vs.* 62·5% and 70%, $P = 0·03$). Nasogastric tubes (27.5% *vs.* 5% and 10%, respectively, $P = 0·03$) and use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (55% *vs.* 25% and 27.5%, respectively, $P = 0·02$) were more common in patients in group 2 compared to patients in groups 1 and 3.

Patients in groups 1 and 2 had fewer concurrent bacterial infections compared to those in group 3 (15% and 32·5% *vs.* 55%, respectively, $P = 0·007$), while this difference between groups 1 and 2 was not statistically significant ($P = 0·15$). The concurrent infections in patients in group 3 developed mainly after the emergence of diarrhoea. Fever was less common in group 1 than in groups 2 and 3 (10% *vs.* 55% and 37·5%, respectively, $P = 0·003$) at the time of positive culture. The difference in cases of hospital-acquired diarrhoea between groups 1 and 2 was not significant ($P = 0·14$).

More patients in groups 1 and 2 received metronidazole than patients in group 3 (58·8%, 60%, 28·2%, respectively, $P = 0·012$); this difference was not significant for vancomycin, which was administered in fewer patients. In addition, there was no significant difference between groups 1 and 2 regarding metronidazole or vancomycin administration. Overall, there were no differences in concomitant antibiotic treatment in the three groups. β-lactam/β-lactamase inhibitors were more commonly administered along with metronidazole or vancomycin in group 1, while linezolid was more commonly administered in group 2.

Outcomes

Six (30%) of the patients in group 1 had severe disease, three of whom had evidence of pseudomembranous colitis on colonoscopy. Overall, two (10%) patients in group 1 required treatment changes because diarrhoea

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persisted; after treatment the diarrhoea resolved. Two patients in group 1 died. None of the deaths was directly attributed to CDI. No significant difference regarding in-hospital mortality was found between the compared groups: 2/20 (10%) in group 1, 5/40 (12·5%) in group 2, and 2/40 (5%) in group 3 ($P = 0{\cdot}49$). Duration of hospitalization was also similar in the three groups (median 9·5, 8·5 and 9 days, respectively).

DISCUSSION

The present study showed that *C. difficile* was infrequently isolated in patients with diarrhoea in a tertiary Greek hospital (6% of specimens sent for possible CDI). Additionally, in patients with cultures positive for *C. difficile*, 14% had also a positive-toxin EIA. Thus, the definition for CDI was met in less than 1% of patients with diarrhoea tested for *C. difficile*. If we consider that the sensitivity of the EIA used for toxin identification in this study is 84–92% [22], then we may hypothesize that the true frequency of CDI in patients with diarrhoea is between 0·95% and 1·03%. None of the observed deaths was directly attributed to CDI. In addition, we found that C+T+ and C+T– patients had more documented infections and received more antibiotics prior the development of diarrhoea than the control group (C–T–). These patients had more concurrent infections but fewer prior infections. This means that most of patients in group 3 developed infections after the emergence of diarrhoea. In addition, due to the retrospective nature of the study, we could not explore whether the diarrhoea in C–T– patients was associated with antibiotics, underlying diseases or concurrent infections. There was no difference between C+T+ patients and C+T– patients regarding prior hospitalization, prior antibiotic treatment and prior infections. Patients in both groups received similar treatment.

In this study we could not address whether the low incidence in definitive CDI was true or was due to unconfirmed cases. A hint towards the second assumption may be the similar therapeutic approach to C+T+ and C+T– cases. In addition, the small but significant increase in the number of ordered tests for CDI during the study period possibly denotes increasing awareness of the burden of the disease in the later years. It should also be noted that in Greece it is not required to test all patients with diarrhoea for CDI, which might also contribute to the low CDI frequency in this study. Under-diagnosis or misdiagnosis is commonly reported for several diseases [23]. Recently, the EUCLID study showed that 23% of CDI cases across 20 European countries, including Greece, were not diagnosed. For Greece the reported undiagnosed cases was as high as 60%. However, only five CDI cases were reported from Greece and the number of missed cases in those not tested at hospitals was similar to the number in all participating European countries [3/56 (5·5%) and 148/2716 (5·4%), respectively] [10].

Lack of awareness regarding the incidence and burden of CDI has been documented in an international survey, which showed that it is higher for European than American authors [24]. We are not familiar with data showing that Greek clinicians may be less aware of the disease than their European colleagues. In the EUCLID study the percentage of samples tested at the participating Greek hospitals for CDI among those submitted for testing in the reference laboratory (52·5%) was lower than the mean of all European countries participating in the survey (62·8%) [10]. This may be an indirect way denoting decreased awareness in Greek doctors, but the limitations of the study should be considered before any firm conclusion is made. Finally, older studies have shown that higher CDI incidence correlates with higher testing rates [25, 26] which may explain the low testing rate in Greek hospitals.

According to a hospital-based survey in Europe the incidence of CDI cases per 10000 patient-days in 2008 ranged from as low as 2·1/10 000 patient-days per hospital in France to as high as 19·1/10 000 in Finland. The reported figure for Greece was 3·7/10 000 (95% confidence interval 1·3–4·9), i.e only Belgium, France, Portugal and Italy had a lower CDI incidence in Western Europe countries [25]. This data was verified in the ECDC survey for hospital-acquired infections (HAIs) for the period 2011–2012, in which gastrointestinal infections accounted for <5% of the HAIs in Greece (55 hospitals), and CDI for <0·5% of HAIs [27]. Although the studies were not performed to compare the incidence of CDI across countries, the outcomes of these surveys are in agreement with this study regarding the low prevalence of CDI in Greece in general and in Crete in particular.

This is the first study from Greece regarding epidemiology of *C. difficile* and clinical characteristics of patients with CDI. In a previous study at the University Hospital of Heraklion (1995–1999) the frequency of CDI in specimens tested for *C. difficile* and all stool specimens was 14·4% and 0·92%, respectively [28]. Although a variation in the frequency of *C. difficile* isolation and CDI during the study period was observed, the data suggests that outbreaks did not occur. The contribution of confounding factors to

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this variation was not studied further. These observations are in contrast to reports from North America and several European countries that documented several outbreaks and an overall increase in the incidence of CDI. In general, CDI seems to be less common in Greece than in several countries of Western Europe [25] and North America [29, 30].

Prior antibiotic treatment and hospitalization are the most important risk factors for CDI. In this study more patients with C+T+ and C+T– specimens received antibiotics prior to the development of diarrhoea than C–T– patients, while prior hospitalization was similar between all three groups. On the other hand, prior use of PPIs, which has arisen as a significant risk factor for CDI [31–34], was more common in C+T– and C–T– patients. We cannot hypothesize that the relatively low frequency of CDI in Crete can be attributed to low antibiotic use, since antibiotic consumption in Greece for both inpatients and outpatients is high [35]. Due to the nature of the study and the small sample size, we were also not able to detect any differences between groups regarding specific types of antibiotics. On the other hand, we can assume that this low frequency of CDI could be attributed to the different types of strains that are prevalent in Greece. For example, the prevalence of the BI/NAP1/027 strain that caused severe outbreaks in North America and some European countries is very low in Greece [25, 35–37]. Finally, it is possible that diarrhoea was due to antibiotic administration for concomitant infections in some of the patients in groups 2 and 3. This assumption could not be explored further due to the retrospective nature of this study.

The diagnosis of CDI depends on a combination of symptoms, signs and diagnostic tests. If EIA is used, up to 25% of cases can be misdiagnosed and guidelines support that the approach to any suspected case with negative-toxin EIA should be individualized based on risk factors and clinical suspicion [11]. The presence of common risk factors for CDI in patients with diarrhoea and C+T– stool samples in an institution where the diagnosis of CDI depends on EIA for toxin identification may explain the high rate of treatment administered for CDI in possibly colonized patients. Furthermore, in settings with low frequency of CDI, such in the University Hospital of Heraklion, the positive predictive value of the EIA becomes even lower, which may have also contributed to overtreatment of C+T– patients.

In-hospital all-cause mortality was 10% for C+T+ patients. Mortality in patients with CDI varied both between published studies and different hospitals or regions in international multicentre studies [25, 38, 39]. In the present study there was no difference in mortality between C+T+ and C+T– patients, but the sample size was small. Other studies that compared symptomatic C+T+ and C+T– patients reported conflicting results. A study in Taiwan reported that no significant difference in mortality was observed between C+T+ and C+T– patients (potential for toxin production was tested by PCR) [40], while another study from the United States reported that both mortality and CDI-related complications were higher in patients with a toxin-positive test [41].

The duration of hospitalization increases in patients with nosocomial infections, including CDI [42]. In this study, there was no significant difference in hospital stay between the compared groups. However, more concurrent infections were noted in groups 2 and 3, which might have been among the factors increasing the duration of hospitalization in these groups. On the other hand, most of patients with definitive CDI did not have severe disease. Finally, due to low sensitivity of EIAs for CDI diagnosis, it is possible that several C+T– patients (the sensitivity of the EIA used in this study according to a systematic review was 84–92%) were false-negative [11, 12, 22].

Almost all *C. difficile* isolates were susceptible to vancomycin and metronidazole. Three isolates with a vancomycin MIC of 2 μg/ml were isolated. Vancomycin-resistant isolates have not been reported in the literature, possibly because breakpoints for *C. difficile* were not available in the previous years. Several studies on *C. difficile* susceptibility used an MIC $\geqslant$16 μg/ml as a cut-off point for vancomycin. However, according to the 2013 EUCAST breakpoints several isolates could be listed as having reduced susceptibility to vancomycin in comparison to wild-type strains. On the other hand, several metronidazole-resistant isolates have been reported, but the rate of resistance did not seem to increase over time [43–45].

The limitations of this study include its retrospective design, the small sample size and the fact that it was performed in one institution over a 7-year period. The fact that the study was performed in a single institution may affect its external validity. However, to our knowledge, this is the first study from Greece that sought to describe the characteristics of patients and outcomes of CDIs. In addition, the outcomes of the study are limited by the use of a less sensitive test for CDI diagnosis (i.e. EIAs instead of toxigenic cultures or nucleic acid amplification tests), which might have led to underestimation of the magnitude of the disease in Crete. In this context, the characteristics and outcomes of included patients

might have been influenced by the decision not to exclude patients with concurrent infections. However, the resolution time of these infections, which was not expected to be short, could not be accurately estimated, since it depended on several factors including host and pathogen characteristics. Finally, the difference in hospital-acquired episodes of diarrhoea, which could have affected the severity of CDI and the outcomes of patients [46, 47] was not significant between C+T+ and C+T– patients.

In conclusion, in this study colonization with *C. difficile* was uncommon. A minority of the cases fulfilled the criteria for the diagnosis of CDI. In addition, the risk factors and outcomes of C+T+ and C+T– patients were similar. Susceptibility of *C. difficile* was similar to that found in other studies. Further multicentre studies are warranted to delineate the characteristics of CDI in Greece.

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DECLARATION OF INTEREST

None.

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