Clostridium difficile infections in South East Scotland

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Clostridium difficile infections in South East Scotland: mortality and recurrence in a region without PCR ribotype 027

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Three hundred and thirty-five patients with laboratory-confirmed *Clostridium difficile* infections (CDIs) were studied for epidemiological features, clinical presentation and laboratory markers. They were followed up for 1 year to determine recurrence and mortality. Four hundred and thirty-two episodes were recorded. One year mortality was 41.8 % of which CDI was listed on 20 % of the death certificates. One year recurrence rate was 22.9 %. PCR ribotype 001 was the commonest epidemiological type and ribotype 027 was not detected. High total leucocyte count and low albumin were significantly associated with mortality, as was the absence of a GI-invasive procedure in the 12 weeks preceding CDI diagnosis, probably due to patients being unfit for the procedure. No association with acid suppressants, deletion in the *tdcC* anti-sigma factor or vancomycin-resistant enterococcus/methicillin-resistant *Staphylococcus aureus* co-infection was detected. One year mortality was higher in patients who developed recurrent infections ($P<0.001$). Differences in ribotype were observed in 2.3 %, 11.11 %, 20 % and 32.4 % isolates with time intervals between sampling of 0–20, 21–40, 41–60 and >60 days, respectively, suggesting that the arbitrary cut-off of 28 days to call a repeat infection a reinfection may not be correct in some cases.

INTRODUCTION

Clinical presentation of *Clostridium difficile* infections (CDIs) can range from mild self-limiting diarrhoea to severe, life-threatening infection, which can be rapidly fatal. Between these two extremes lie disease syndromes, including abdominal pain, fever and leucocytosis, pseudomembranous colitis, bowel perforation, sepsis and shock. A characteristic feature of *C. difficile* is its propensity to cause recurrent infections, which can increase the length of stay and overall cost of hospitalization (Spencer, 1998). The predisposing factors associated with recurrent CDI have not yet been accurately determined, although a number of studies have investigated the subject. Some of these suffered from paucity of numbers and hence lacked the power to detect significant differences between recurrent and non-recurrent cases. This present study aimed to evaluate the incidence, clinical features and molecular characteristics of recurrent CDI in an area without ribotype 027.

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Abbreviations: CA, community-associated infection; CDI, *Clostridium difficile* infection; CCI, Charlson co-morbidity index; GDH, glutamate dehydrogenase; HA, hospital-associated infection; HCA, healthcare-associated infection; HCF, health care facility.

Early identification of patients who are at high risk for severe CDI may help clinicians to alter the modifiable factors and hence improve outcomes. However, there is a great deal of variation in the criteria used to study adverse outcomes. Studies have used 30 day or 90 day all-cause mortality, colonic surgery, pseudomembranous colitis or various combinations of these as predictors of adverse outcome. The most common end point used is mortality up to 30 days from diagnosis (Bloomfield *et al.*, 2012).

Predictors of severity such as total leucocyte count, levels of serum albumin, serum creatinine and CRP, age, co-morbidities, length of hospital stay and immunosuppression have been studied. However, two recent systematic reviews have concluded that the studies published so far have several potential limitations like retrospective design, poor sample size, single stage stool testing, and absence of multivariable analyses to adjust for confounders (Abou Chakra *et al.*, 2012; Bloomfield *et al.*, 2012).

The prevalence of epidemiological types of *C. difficile* varies geographically but the recent increased incidence and mortality has often been attributed to the suspected hypervirulence of PCR ribotype 027. It is widely believed that ribotype 027 can cause severe infections and higher overall 30 day mortality than other ribotypes (Goorhuis

et al., 2011). However, in a Europe-wide, hospital-based survey infection by ribotypes 018 and 056 was significantly associated with complicated disease outcome (Bauer *et al.*, 2011).

This report describes a prospective study of mortality and recurrence in an area where PCR ribotype 027 has been absent. The incidence, clinical features and molecular characteristics were studied with the aim to determine whether the predisposing factors are different from other studies performed in regions where PCR ribotype 027 has been found.

METHODS

Patients. Patients whose stool samples were sent routinely to the diagnostic microbiology laboratory of the Royal Infirmary of Edinburgh from August 2010 to July 2011 and which tested positive for *C. difficile* toxin by two-step glutamate dehydrogenase (GDH) and toxin A/B ELISA (see later) were prospectively followed up for 1 year after the first stool sample tested positive. Relevant ethical approval was obtained for the study.

Data collection. Initial patient demographics, clinical features and co-morbidity data were collected at the start of each episode of CDI, at 10 days and at 1 year. For outpatients, the same data were collected by telephone contact with the requesting doctor. Recovery, recurrence, treatment, and mortality were recorded. The Charlson co-morbidity index (CCI) was calculated using a macro enabled Excel tool available online (Hall *et al.*, 2004). Haematological and biochemical data (white cell count, creatinine, lactate) were obtained from the local laboratory information system. Mortality data were obtained from the NHS Lothian Public Health Department. Cause of death was determined by reviewing the ICD codes on the death certificates. ICD10 codes A04.7, A09, A41.4, A49.8 were included in analysis (Anonymous, 2006).

Stool samples were tested using a two-step algorithm for *C. difficile* toxin EIA (A and/or B) (Tox A/BII, Techlab) and GDH (C.DIFF CHEK-60, Techlab). GDH-negative stools were excluded from the study. The stools positive for both GDH and toxin EIA were cultured on cefoxitin-cycloserine-egg yolk (CCEY) medium (Brazier, 1993). All isolates were later subjected to toxin A and toxin B PCR (Persson *et al.*, 2011).

PCR ribotyping and detection of gene deletion. All DNA templates were prepared by the Chelex DNA extraction method (Stubbs *et al.*, 1999). Previously published (Persson *et al.*, 2011) toxin gene primers (FAM labelled) were used in a multiplex format though the *cdtA* was omitted, as it was the least informative. PET labelled O'Neill primers were used for ribotyping (O'Neill *et al.*, 1996). The products of these PCRs were mixed in highly deionized (HiDi) formamide (8 µl HiDi Liz 600 in 500 µl formamide buffer) and analysed by capillary electrophoresis. The toxin multiplex and PCR ribotyping protocols were initially run on the same thermocycling protocol: initial denaturation at 95 °C for 5 min, 25 cycles of 95 °C for 60 s, 55 °C for 60 s and 72 °C for 90 s, final extension at 72 °C for 5 min. However, since the initial reactions for ribotyping were rather weak, the cycle for ribotyping was later increased to 27 cycles. After PCR, the products of the ribotyping and toxin gene multiplex were mixed as follows. First dilution: 18 µl nuclease-free water and 10 µl ribotyping PCR products and 2 µl toxin gene PCR product. This was mixed well by pipetting. Final dilution, 1 µl of the diluted products were mixed with 9 µl of HiDi formamide mix.

Capillary gel electrophoresis (ABI 3730) was used for PCR product analysis and ribotypes were assigned by comparing with a set of known strains on GeneMarker® software version 1.95 (Softgenetics). Known strains of ribotypes 001, 106 and 027 were included with each run of ribotyping and toxin multiplex PCR for quality control. The toxin gene images were compared against the PaLoc indicator panel (kindly provided by Dr Derek Fairley).

Clinical case definitions. Clinical case definitions were adapted from Society for Healthcare Epidemiology of America (SHEA) and Health Protection Agency (HPA) guidelines (HPA, 2008; McDonald *et al.*, 2007). A case was defined as a patient with diarrhoea where the stool takes the shape of the container (grade 5–7 as per Bristol stool chart) or toxic megacolon with stool positive for *C. difficile* toxin A and/or B without other known aetiology (McDonald *et al.*, 2007). An episode of CDI was defined as a case with the criteria above who remains continuously symptomatic with a break of less than 48 h in symptoms attributable to CDI. A repeat episode was defined as another episode of CDI, which occurred after the duration of treatment of the initial episode, with at least 48 h of an intervening asymptomatic period.

Hospital-associated infection (HA-CDI) was defined as CDI that developed after 48 h of admission into a health care facility (HCF), community-associated infection (CA-CDI) was defined as CDI that developed in a patient with no history of health care contact in the 12 weeks prior to diagnosis. Patients who were admitted in nursing homes (without admission to a hospital in the past 12 weeks) and those who had contact with a hospital in the past 4–12 weeks (indeterminate category by SHEA definitions) were included in the CA-CDI category.

Healthcare-associated infection (HCA-CDI) was defined as CDI developing in a patient within 4 weeks of contact with a HCF but less than 48 h after admission to a HCF. For the purpose of analyses, this was included in the HA-CDI category.

Immunosuppression was defined as the presence of one or more of the following conditions: acquired immunodeficiency syndrome (AIDS), solid organ or haematopoietic stem cell transplant, neutropenia, immunosuppressive drug or systemic corticosteroids for >1 month, corticosteroid >10 mg or chemotherapy in last 2 months.

Major gastrointestinal (GI) procedure was defined as an invasive procedure involving instrumentation, handling or surgical exploration of the GI tract under anaesthesia. GI pathology was defined as inflammatory, structural or neoplastic condition of the GI tract.

A patient was considered colonized with methicillin-resistant *Staphylococcus aureus* (MRSA) or vancomycin-resistant *Enterococcus* (VRE) if electronic records stated that either of these had been isolated from the patient any time in the past before the episode of CDI.

For statistical analyses of mortality, all patients who died with CDI listed on their death certificates 1 year from diagnosis ($n=28$) were compared to those who were alive at 1 year or died due to causes unrelated to the CDI ($n=299$). Thirty day all-cause mortality ($n=63$) was also compared to those who were alive at 30 days ($n=264$).

For recurrent infections, patients who had a documented episode in the past before the commencement of this study ($n=35$) were removed from analysis. In addition, to exclude those patients who die before they get a chance to develop recurrence, all patients who died within 30 days of development of CDI ($n=53$) were also removed from this analysis. Hence, a total of 247 patients were included in the statistical analysis of this section (56 patients who developed more than one episode and 191 patients who had only one documented episode). Statistical analysis was performed using Minitab statistical

software version 15.1.0.0. Fisher's exact test and the chi-square test were used for categorical variables and the two-sample *t*-test for numerical variables. Multivariate analyses were performed using binary logistic regression analysis.

RESULTS AND DISCUSSION

Four hundred and twelve samples from 345 patients (corresponding to 432 clinical episodes) were positive with GDH and toxin ELISA combination testing. Of these, 10 samples were from patients under 2 years of age. Since the significance of toxin positivity from this age to exclude group is not clear, these were removed from further analysis. Hence, 402 samples corresponding to 432 clinical episodes were analysed.

The majority of samples were obtained from geriatric subjects. The age distribution of the patients ranged from 2 to 97 years (mean age of 73 years). Of the 335 patients, 2.6 % were under 18 years of age, 24.2 % from 18 to 60 years and the remaining 73.1 % were over 60 years of age. This reflects the trend in previous years (Reddy *et al.*, 2010).

The CCI was available for 333 patients. Although the unadjusted version was also recorded, the age-adjusted version was used in the analyses. In this study, 19.2 % patients had a CCI score of 0. The use of acid-suppressing medication was found in 51.3 % patients and GI pathology was found in 55.5 % patients. A total of 19.1 % patients underwent a GI invasive procedure in the 12 weeks preceding development of CDI and 57 patients in the study were deemed to be immunosuppressed.

Results of statistical analyses are given in Tables 1–6.

Analysis of mortality

PCR ribotype. A recent study in the Netherlands followed up 1350 cases of CDI of which 177 patients died within 30 days and 497 patients had died 1 year after diagnosis, accounting for a mortality risk of 13.1 % and 36.8 %, respectively (Hensgens *et al.*, 2013). In the quoted study 20.8 % of those with known ribotype 027, 15.8 % with known ribotype 001 and 14.8 % with ribotype 078 died within 30 days of diagnosis (PCR ribotypes were not available for 49 % of patients). Ribotype information was available for only 25 patients who had CDI-related codes on their death certificates, of which 24 % were infected with ribotype 078, 12 % with ribotype 045 and 12 % with ribotype 001. Eight percent of all isolates were ribotype 027. Comparatively, in the present study, of the 335 patients, 63 died within 30 days of diagnosis (18.8 %) and 140 (41.79 %) were deceased within the 1 year of follow-up. In addition, 21.7 % with known PCR ribotype 001 and 21.4 % with known ribotype 078 died within 30 days of diagnosis. No isolates with PCR ribotype 027 were detected in the study. Data were not available for eight patients.

Table 1. Bivariate analysis of continuous variables associated with death from CDI (as listed on the death certificate) within 1 year of the index episode, and 30 day all-cause mortality

Variable	1 year CDI-related mortality				Absence of CDI-related mortality			*P*-value	30 day all-cause mortality				Alive at 30 days				*P*-value
	n	Mean	SD	SEM	*n*	Mean	SD	SEM		*n*	Mean	SD	SEM	*n*	Mean	SD	SEM	
Age (year)	28	77.5	12.9	2.4	299	66.5	21.4	1.2	0.000	63	78.6	11.8	1.5	264	64.8	21.9	1.3	0.000
CCI (age adjusted)	28	5.5	2.67	0.51	298	4.76	3.24	0.19	0.176	63	6.41	2.77	0.35	263	4.44	3.18	0.20	0.000
Antibiotics given 8 weeks preceding onset (no.)	25	1.60	1.29	0.26	270	1.91	1.33	0.081	0.226	54	1.76	1.23	0.17	241	1.91	1.35	0.087	0.430
Episodes within 1 year of study (no.)	28	1.357	0.559	0.11	299	1.284	0.678	0.039	0.522	63	1.222	0.522	0.066	264	1.307	0.698	0.043	0.284
Leucocyte count (cells $\times 10^9\ l^{-1}$)	26	19.9	11.6	2.3	246	12.70	7.45	0.48	0.005	53	16.9	11.2	1.5	219	12.54	7.07	0.48	0.009
Serum albumin level at onset ($g\ l^{-1}$)	22	25.23	5.87	1.3	190	29.79	6.84	0.50	0.002	42	27.07	5.94	0.92	170	29.88	7.0	0.54	0.010

Table 2. Bivariate analysis of categorical variables associated with death from CDI (as listed on the death certificate) within 1 year of the index episode, 30 day all-cause mortality and 1 year CDI-related mortality

Variable		Total (no.)	CDI-specific mortality within 1 y of index episode (no.)	No CDI-specific mortality within 1 y of index episode (no.)	*P*-value*	30 day all-cause mortality (no.)	Alive at 30 day (no.)	*P*-value*
Gender	Male	118	10	108	0.966	22	96	0.830
	Female	209	18	191		41	168	
Immunosuppression	Absent	273	23	250	0.793	57	216	0.096
	Present	54	5	49		6	48	
Place of acquisition	CA	83	11	72	0.077	20	63	0.205
	HA	244	17	227		43	201	
Acid suppressants (PPI and H2 receptor antagonists)	Given	169	11	158	0.253	27	142	0.425
	Not given	128	13	115		25	103	
Underlying GI pathology	Not known to be present	180	14	166	0.575	40	140	0.134
	Present	147	14	133		23	124	
Deletion detected in *tcdC*	Absent	253	19	234	0.019	50	203	0.969
	Present	50	9	41		10	40	
Presence of *cdtB*	No	265	21	244	0.037	51	214	0.521
	Yes	38	7	31		9	29	
Colonization with MRSA or VRE prior to CDI	No	263	20	243	0.209	47	216	0.195
	Yes	64	8	56		16	48	
Invasive GI procedure in 12 weeks prior to CDI	No	265	27	238	0.011	58	207	0.013
	Yes	62	1	61		5	57	
Antibiotics given 8 weeks prior to CDI†	No	33	4	29	0.329	7	26	0.694
	Yes	272	21	251		50	222	
Ribotype	078	27	5	22	0.881	6	21	0.741
	Non-078	276	23	253		54	222	

*Pearson's chi-squared or Fisher's exact test.

†Individual antibiotics (penicillin V, piperacillin-tazobactam, amoxicillin, amoxicillin-clavulanic acid, flucloxacillin, clindamycin, macrolides, gentamicin, tetracycline, meropenem, cotrimoxazole, nitrofurantoin, quinolones, 2nd and 3rd generation cephalosporins, glycopeptides, rifampicin, linezolid, metronidazole) were also compared but no statistically significant difference was found.

Table 3. Binary regression model for multivariable analysis of factors associated with CDI-related mortality within 1 year of index episode

Variable	*P*-value	Odds ratio	95 % CI	
			Lower limit	Upper limit
Age	0.147	1.03	0.99	1.06
Presence of *cdtB*	0.5	1.99	0.27	14.59
Presence *tcdC* deletion	0.483	0.51	0.08	3.31
GI procedure within 12 weeks preceding index episode	0.026	0.06	0.01	0.72
Leucocyte count (cells $\times 10^9$ l^{-1})	0.016	1.07	1.01	1.13
Serum albumin level at onset (g l^{-1})	0.014	0.90	0.82	0.98

Distribution of ribotypes in the patient group is given in Fig. 1 and time to death stratified by information on the death certificates is given in Fig. 2.

There have been suggestions that the ribotype of a *C. difficile* isolate may determine its potential to cause severe disease (Arvand *et al.*, 2009; Baldan *et al.*, 2010). However, in keeping with other multivariate clinical studies (Walk *et al.*, 2012; Wilson *et al.*, 2010), this study suggests that mortality is not related to ribotype whereas there is a positive correlation with total leucocyte count and serum albumin (Tables 1 and 3). As is apparent from Fig. 1, there is heterogeneity within this population and no particular ribotype is over-represented. Ribotyping does however, provide a method for classifying *C. difficile* isolates, which is often required for epidemiological purposes and is a starting point for comparative studies.

Differences in toxin genes *tcdC* and *cdtB*. The presence of the binary toxin gene (*cdtB*), the major in-frame deletions (18, 39 and 54 bp), the truncating 1 bp deletion at position 117 and a C to T mutation at position 184 in the toxin suppressor gene (*tcdC*: anti-sigma factor) was compared and a significant difference was found between the presence of a deleted *tcdC* (P=0.019,) and presence of the binary toxin gene (P=0.037) between patients with and without CDI-specific 1 year mortality as shown in Table 2. However, this difference was not found in the multivariable regression model (Table 3). It has been suggested previously that these mutations are associated with increased virulence (Carter *et al.*, 2011; Knetsch *et al.*, 2011). However, doubts exist as to the significance of gene deletions (Curry *et al.*, 2007) and clinical studies have failed to find a correlation between their presence and disease outcome (Verdoorn *et al.*, 2010; Goldenberg & French, 2011). The latter study did, however, suggest an association between the presence of the binary toxin genes, increased total leucocyte count and all-cause mortality at 30 days.

Age and gender. Age was significantly associated with mortality in both groups in the bivariate analysis (Table 1) but only with 30 day all-cause mortality in the multivariable model. Past studies have reported conflicting results. A study compared 82 patients who survived and 46 patients who died within 30 days of CDI and did not find a significant difference in age (>75 years) or gender (Wilson *et al.*, 2010). Conversely, an American study compared the 30 day mortality due to CDI and found age >80 years significantly associated with mortality; however, there was no such association with gender (Morrison *et al.*, 2011).

Co-morbidities. The CCI was significantly greater in those with 30 day mortality but not in the 1 year CDI-specific mortality (Table 1). This association was not statistically significant in the multivariable analyses (Tables 3 and 4). A German study analysed factors likely to predict severe CDI (defined as profuse diarrhoea associated with a heart rate beats pm/systolic blood pressure mm Hg >1.5 at initial diagnosis) and concluded that CCI was independently associated with the risk, along with C-reactive protein (Hardt *et al.*, 2008). Other studies that have used the CCI have also concluded that it is significantly different between the two groups, although the end point used to define

Table 4. Binary regression model for multivariable analysis of factors associated with 30 day all-cause mortality

Variable	*P*-value	Odds ratio	95 % CI	
			Lower limit	Upper limit
Age (y)	0.014	1.03	1.00	1.06
GI procedure within 12 weeks preceding index episode	0.020	0.25	0.08	0.80
Leucocyte count (cells $\times 10^9$ l^{-1})	0.004	1.06	1.02	1.11
Serum albumin level at onset (g l^{-1})	0.086	0.95	0.89	1.01

Table 5. Bivariate analysis of continuous variables compared between the patients with recurrent and non-recurrent CDI

Variable	Recurrent CDI				Non-recurrent CDI				*P*-value*
	n	Mean	SD	SE mean	*n*	Mean	SD	SE mean	
Age (y)	56	69.9	18.8	2.5	191	62.8	22.5	1.6	0.020
CCI (age adjusted)	56	4.86	2.77	0.37	189	4.11	3.2	0.23	0.089
Antibiotics given 8 weeks preceding onset (no.)	50	2.18	1.44	0.20	175	1.79	1.29	0.098	0.092
Leucocyte count (cells $\times 10^9$ l^{-1})	49	13.8	7.47	1.1	155	12.21	7.33	0.59	0.198
Serum albumin level at onset (g l^{-1})	35	28.11	5.7	0.97	123	30.49	7.13	0.64	0.045

*Two-sample *t*-test without assuming equal variances.

severity and the cut-off score used vary between the studies (Das *et al.*, 2010; Labbé *et al.*, 2008; Cadena *et al.*, 2010).

The CCI score includes various co-morbidities and is generally used as an indicator to assess the risk of dying within 1 year. Since it includes various chronic conditions, it is useful as a combination indicator negating the need to assess each co-morbidity independently. However, since it involves the analysis of 19 different parameters its practical application as a routine marker of severity is difficult.

Table 6. Bivariate analysis of categorical patients between patients who developed recurrent and non-recurrent CDI

Variable		Recurrent CDI (no.)	Non- recurrent CDI (no.)	Total (no.)	*P*-value*
Gender	Male	19	67	86	1.000
	Female	37	124	161	
Immunosuppression	Present	7	37	4	0.321
	Absent	49	154	203	
Place of acquisition	CA	18	42	60	0.155
	HA	38	149	187	
Antacids (PPI and H2 receptor antagonists)	Present	31	94	125	0.334
	Absent	19	82	101	
Underlying GI pathology	Present	28	80	108	0.288
	Absent	28	111	139	
Deletion detected in *tcdC*	Present	6	32	4	0.295
	Absent	48	144	192	
Colonization with MRSA or VRE prior to CDI	Present	12	39	59	0.853
	Absent	44	151	195	
GI-invasive procedure in 12 weeks prior to CDI	Performed	9	47	56	0.207
	Not performed	47	144	191	
Use of tazocin in 8 weeks preceding illness	Yes	20	54	74	0.234
	No	30	122	152	
Severity of first episode	Non-severe	28	109	137	0.281
	Severe	27	75	102	
Antibiotics given 8 weeks prior to CDI	Yes	49	156	205	0.078
	No	2	24	26	
Presence of *cdtB*	Present	6	23	29	0.818
	Absent	48	153	201	
Ribotype	001	12	17	29	0.024
	078	2	18	20	
1 year mortality from CDI	Yes	9	1	10	0.000
	No	46	183	229	

*Fisher's exact test.

Antibiotics. Antibiotics disrupt the normal flora of the gut allowing *C. difficile* to proliferate and cause CDI. In the present study administration of antibiotics in the 8 weeks preceding CDI onset was compared but there was no statistical difference found. Each individual antibiotic was also analysed separately but no statistically significant difference was found (Table 2). The use of antibiotics has been studied in the past but an American study (Morrison *et al.*, 2011) also did not find a statistical correlation between their use and 30 day mortality.

Total leucocyte count and serum albumin. Total leucocyte count was found to be statistically significant in both bivariate and multivariate analyses (Tables 1, 3 and 4) though serum albumin was not significant in the multivariable model of 30 day all-cause mortality. Previous studies have examined the association of these markers using cut-off values varying from >15 cells $\times$ 10^9 l^{-1} to >30 cells $\times$ 10^9 l^{-1} for total leucocyte count and from <20 g l^{-1} to <30 g l^{-1} for albumin, some also having used mean values (Dharmarajan *et al.*, 2000; Gujja & Friedenberg, 2009; Andrews *et al.*, 2003; Pépin *et al.*, 2007). Although the criteria for outcome varied between the studies, a recent meta-analysis found that both these parameters were useful markers of severity to determine outcome (Bloomfield *et al.*, 2012).

Invasive GI procedures and underlying GI pathology. There was a negative correlation of the performance of an invasive GI procedure with death in both analyses. The reason for this paradoxical phenomenon is not clear but may be due to patients being generally more unfit to undergo a GI procedure and hence more likely to die than those who are considered fit to undergo GI invasion. The presence of an underlying GI pathology was not different between the groups studied.

Immunosuppression. The definition of immunosuppression is not standardized and hence it is difficult to compare directly the results of this study with those of others. This may also be a reason why there is heterogeneity in the reported significance of immunosuppression as a predictor of mortality. However, a large study comparing three groups of patients with CDI (without steroids), CDI with steroids and a non-CDI control arm suggested that the use of any glucocorticoid resulted in an increased mortality with a hazard ratio of 2.1 ± 0.19 ($P<0.001$) comparing all patients who had CDI. Mortality at 30 days was higher in the CDI with steroids group as compared to those without steroids (19.3 vs 9.6 %) (Das *et al.*, 2010). Hence, it is likely that immunosuppression is related to adverse outcome in CDI. This was however, not detected in the present study.

Hospital-associated acquisition and prior colonization with MRSA/VRE. These factors were not found to be significantly associated in any of the above statistical comparisons of mortality. Since the definition of hospital acquisition has only recently been standardized, the studies that have examined this variable have used a variety of definitions.

Antacids (including proton-pump inhibitors). A statistically significant correlation was not found in the use of acid suppressant medication and the development of severe CDI in any of the above analyses.

Analysis of recurrent infections

In the present study, 19.4 % of patients experienced more than one episode (recurrence or relapse) in the 1 year follow-up period. After removal of patients who had a documented episode in the past ($n=35$) and all patients who died within 30 days of development of CDI ($n=53$) the recurrence rate was 22.9 %. The incidence of recurrent CDI has been reported to range from 15 to 35 % of cases (Fekety *et al.*, 1997; Barbut *et al.*, 2000).

Total leucocyte count and serum albumin. The present study did not find an association between total leucocyte count and recurrence; however, the albumin levels appeared to be statistically significant (Table 5). Choi *et al.* (2011) reported that white blood cell count, serum albumin and C-reactive protein at diagnosis were not significantly different between the recurrent and non-recurrent groups. Eyre *et al.* (2012) studied 12 biomarkers in their study group but only higher C-reactive protein level and higher neutrophil count at first CDI independently appeared to increase the recurrence risk.

Patients with recurrences were also more likely to die within 1 year from the first episode as compared to those without recurrent disease. Interestingly, in our study, ribotype 001 was associated with more recurrent disease than ribotype 078 (Table 6).

Association with antibiotics. CCI, administration of antibiotics 8 weeks prior to the first episode and the total number of different antibiotics given prior to CDI were also trending towards a statistically significant value (*P*-values of 0.089, 0.078 and 0.092, respectively).

In the same study mentioned previously (Choi *et al.*, 2011), patients who received more than three antibiotics were more common in the recurrent group as compared to the non-recurrent group, but this association was not found after adjusting for confounders.

Gastric acid-suppressing agents. No statistically significant difference ($P=0.334$) was found between the use of acid-suppressing agents (proton-pump inhibitors, and H2-receptor antagonists) in the two groups analysed in the present study. This reflects the findings of a previous study examining recurrent disease and the use of these agents (Choi *et al.*, 2011). However, a study by Tal *et al.* (2002) did find the use of H2 receptor antagonists to be a

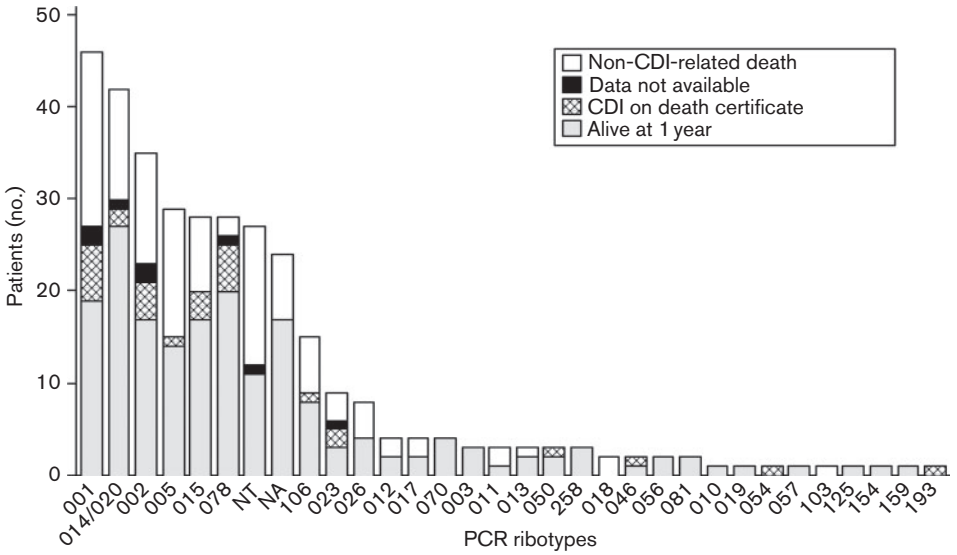

Fig. 1. Cause-specific mortality. NT, not typable; NA, sample not available.

statistically significant predictor of recurrent CDI ($P \leqslant 0.02$).

Immunosuppression. Although previous studies have reported recurrent CDI associated with allogeneic stem cell transplant (Chang *et al.*, 2012) and GI graft versus host disease (Alonso *et al.*, 2012), the lower power in these studies did not allow this factor to be studied in detail. A higher-powered recent analysis did not find a statistically significant correlation between dialysis or chemotherapy and development of recurrent CDI (Eyre *et al.*, 2012). In keeping with this finding, the present study did not find a correlation between immunosuppression and risk of CDI.

Other hospital-associated pathogens. A retrospective cohort of 84 patients with CDI found enteric colonization with VRE to be statistically correlated with the risk of developing recurrent CDI (Choi *et al.*, 2011). The reason for this phenomenon is unclear though since VRE and *C. difficile* are both recognized hospital-associated pathogens, the co-infection could be due to shared risk factors rather than a direct causative role. In the present study, the effect of colonization with either VRE or MRSA was evaluated (Table 6) but not found to be statistically significant. Eyre *et al.* (2012) also studied the effect of prior MRSA colonization and it appeared to be a possible factor in the univariable analysis but they did not find it a

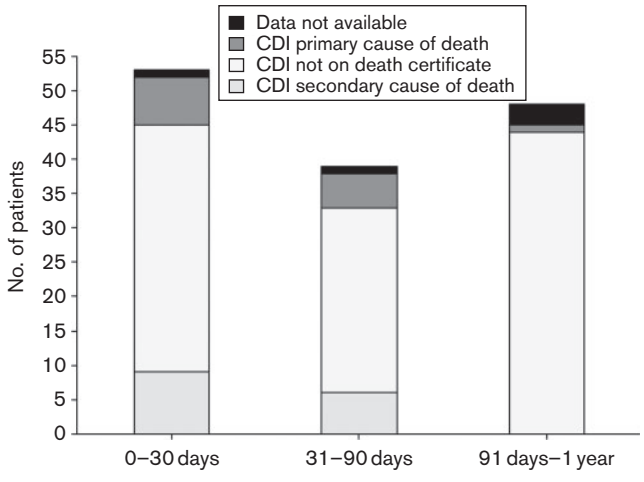

Fig. 2. Time to death stratified by cause of death. Time to death was calculated from the last recorded episode of CDI.

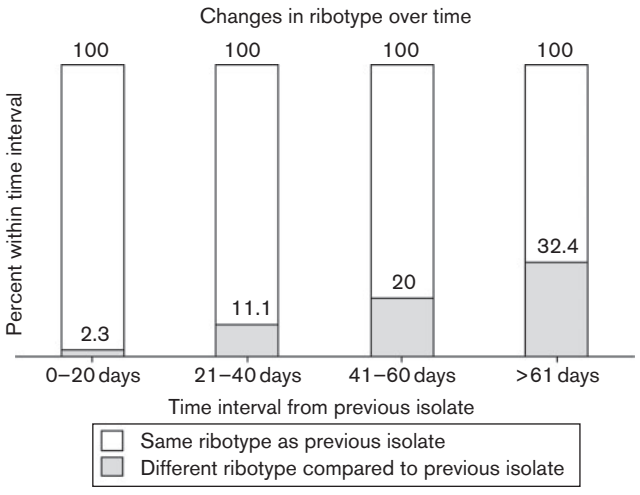

Fig. 3. Differences over time in ribotype of isolates compared to previous isolates.

statistically significant predictor on the multivariable model.

Molecular analysis of recurrences

Differences in ribotype were observed in 2.3 %, 11.11 %, 20 % and 32.4 % of isolates with time intervals between sampling of 0–20, 21–40, 41–60 and >60 days, respectively (Fig. 3). This chart demonstrates a steady rise in the proportion of isolates, which were different as compared to the previous ribotype isolated from the same patient when plotted against time. In the column 0–20 days, there was one isolate at 16 days, in column 21–40 days, there were three isolates at 23, 26 and 27 days.

This result suggests that the arbitrary cut-off of 28 days to call a repeat infection a reinfection may be correct in the majority of cases where the ribotype is different; however, there are some cases (four in this study) where there is a different ribotype isolated even before 28 days. This must be kept in mind if a patient presents again with symptoms after a period of recrudescence or if the nature of illness changes from mild to severe, since this may be due to a reinfection.

On the other hand, there are a number of isolates obtained from the same patient even after 28 days where the ribotype is the same as the previous isolate. This suggests that even though a patient may present with a fresh set of symptoms after 28 days it may be the same episode and hospitals should not be considered liable for this new infection if it is the same strain being carried by the patient. If this new set of symptoms occurs in hospital, it is likely to be due to antibiotic usage or due to other predisposing factors, which may or may not be modifiable.

An earlier study (Wilcox *et al.*, 1998) defined recurrence of *C. difficile* diarrhoea as the resumption of symptoms, after cessation for at least 3 days, with laboratory-confirmed cytotoxin-positive faeces. They analysed *C. difficile* isolates by RAPD analysis and found that 56 % of clinical recurrences of infection are in fact due to reinfection as opposed to relapse.

In a study comprising *C. difficile* isolates from 102 patients with repeat episodes of CDI (Kamboj *et al.*, 2011), it was found that in those patients who had a second episode within 2–4 weeks of the original episode 90 % had the same ribotype, those who had a second episode 4–8 weeks apart had the same ribotype in 86.5 % of cases and episodes separated by >8 weeks had the same ribotype in 65 % of cases. Results from our study are roughly comparable with this recent study.

Another study that compared strains by serotyping from repeat episodes of CDI from patients found two different serogroups in 21.5 % of patients and the same serogroup in 78.5 % of cases. PCR ribotyping was used to discriminate the latter group and showed a different pattern in 65.7 % of cases. Their results suggest that 45 of 93 (48.4 %) clinical recurrences were in fact due to reinfections with a different strain. Delay of relapse and reinfection had a median of 28 and 38 days, respectively (Barbut *et al.*, 2000).

This study adds to the existing knowledge of these complications of CDI. Characteristics of CDI-related mortality and recurrence seen in this region of Scotland have been documented and the effects of potential predisposing factors analysed. Compared to studies in which ribotype 027 has been reported in an endemic situation without major outbreaks, there do not appear to be any noteworthy differences.

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