Impact of *Clostridium difficile* infection: clinical and economic perspectives

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

by

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Declaration

This thesis is the result of my own work. The material contained within this thesis has not been presented, nor is currently being presented, either wholly or in part for any other degree or qualification.

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This research was carried out in the Department of Molecular and Clinical Pharmacology, in the Institute of Translational Medicine, at the University of Liverpool.

“Educação não transforma o mundo.

Educação muda as pessoas.

Pessoas transformam o mundo”.

(Paulo Freire)

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Abbreviations

AIDS	acquired immune deficiency syndrome
APIC	Association of Professionals in Infection Control and Epidemiology
AWMSG	All Wales Medicines Strategy Group
Bca	bias corrected and accelerated
CAI	community-acquired infection
CCI	Charlson comorbidity index
CDC	Center for Disease Control and Prevention
CDI	*Clostridium difficile* infection
CDT	*C. difficile* transferase
CEA	cost-effectiveness analysis
CF	cephalosporin
CHEERS	Consolidated Health Economic Evaluation Reporting Standards
CI	Confidence interval
CIP	ciprofloxacin
CLI	clindamycin
COI	Cost of Illness
CRF	Case Report Form
CRP	C-reactive protein
CTT	cefotetan
CUA	cost utility analysis
DALY	disability-adjusted life years
ECDC	European Centre for Disease Prevention and Control
eGFR	estimated glomerular filtration rate
EIA	enzyme immunoassay
EPMA	End-to-End E-Prescribing & Medicines Administration
EQ-5D-3L	EuroQol five dimension questionnaire
EQ-VAS	EuroQol visual analogue scale
ERY	erythromycin
exp	exponential
F	frequency

FDA	Food and Drug Administration
FDX	fidaxomicin
FMT	faecal microbiota transplant
FOX	cefoxitin
FQ	fluoroquinolones
GBP	British pounds
GDH	glutamate dehydrogenase
GLM	generalized linear model
HCAI	healthcare-associated infection
HIV	human immunodeficiency virus
HPA	Health Protection Agency
HR	hazard ratio
HRG	Healthcare Resource Group
HROOL	health-related quality of life questionnaire
IBD	inflammatory bowel disease
ICD	International Classification of Diseases
ICE	Integrated Clinical Environment
ICER	incremental cost-effectiveness ratio
IDB	Infectious Diseases Biobank
IDSA	Infectious Diseases to Society of America
IMD	index of multiple deprivation
iPM	Patient Manager
IQR	interquartile range
LCL	Liverpool Clinical Laboratories
LoS	length of stay
LSOA	Lower layer Super Output Area
M	median
MIC	minimum inhibitory concentration
MRSA	methicillin resistant Staphylococcus aureus
MTZ	metronidazole
NAAT	nucleic acid amplification test
NHS	National Health Service

NICE	National Institute for Health and Care Excellence
NPV	negative predictive value
NTCD	non-toxigenic *C. difficile*
OR	Odds ratio
p	p-value
P	Percentage
PCR	polymerase chain reaction
PCT	Procalcitonin
PHE	Public Health England
PLICS	patient-level information and costing system
PPE	personal protective equipment
PPI	proton-pump inhibitors
PPV	positive predictive value
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
PROSPERO	International Prospective Register of Systematic Reviews
QALY	quality-adjusted life years
RCDI	recurrent CDI
RLBUHT	Royal Liverpool and Broadgreen University Hospitals Trust
RT027	NAP1/BI1/027
SE	standard error
SHEA	Society for Healthcare Epidemiology of America
SMC	Scottish Medicines Consortium
SSI	surgical site infection
tcdA	enterotoxic toxin A
tcdB	cytotoxic toxin B
TOX	toxin enzyme immunoassays
tpi	triose phosphate isomerase
UK	United Kingdom
US	United States
VAN	vancomycin
WCC	white cell count
WHO	World Health Organization

Publications and communications

Poster presentations

Nakamura CA, Roberts P, Mcgregor K, Carneiro L, Hekmat Z, Richards H, Wiltshire A, Neal T, O'Brien S, Pirmohamed M, Beadsworth M, Miyajima F: An evaluation of the toxigenicity of *Clostridium difficile* isolates and clinical outcomes from GDH-positive specimens in a large hospital setting. ASM Microbe, 1-5 June 2017, New Orleans. United States.

Nakamura CA, Roberts P, Beadsworth M, O'Brien S, Pirmohamed M, Hughes D, Miyajima F: Health-economic evaluation of *Clostridium difficile* infection (CDI) and epidemiology in England and Merseyside. ISPOR 18th Annual European Congress, 7-11 November 2015, Milan, Italy.

Miyajima F, Swale A, Roberts P, **Nakamura CA**, Little M, Beeching N, Beadsworth M, Farragher T, Parry C, Pirmohamed M: Predicting poor disease outcomes of *Clostridium difficile* infection: the quest for more answers? 5º International *Clostridium difficile* Symposium (ICDS), 19-21 May 2015, Bled, Slovenia.

Abstract

Clostridium difficile infection (CDI) is one of the most common causes of infective hospital-acquired diarrhoea and one of the leading causes of healthcare-associated infection (HCAI) worldwide. The emergence of hypervirulent strains has caused outbreaks in several countries, and the disease has been a challenge to healthcare workers, settings and systems mainly related to the disease heterogeneity, high rates of recurrence, antibiotic resistance and high disease-associated healthcare costs.

In this thesis, a CDI cohort recruited over different time periods (2008-2012 and 2013-2015) was used. More patients were recruited by a clinical audit (2008-2012 and 2012-2016) to increase sample size for some of my analyses, and to assess the representativeness of the cohort group. In general, the cohort and audit groups were similar, but did have some notable differences: audit patients were older [79 vs 75 years (IQR: 61-81), p<0.001 for phase I and 77 vs 66 years (IQR: 56-79), p=0.007 for phase II], and more debilitated as mortality rates were higher, considering both short-term mortality (32% vs 7%, p<0.001, for phase I and 25% vs 4%, p<0.001, for phase II) and long-term mortality (62% vs 32%, p<0.001, for phase I and 59% vs 41%, p=0.010, for phase II).

Taking all patients from 2012 to 2016 into consideration, carrier patients (GDH+/TOX-/PCR+) and CDI cases were more likely to have had longer hospitalisation [(HR=0.73, 95% CI: 0.59-0.90) and (HR=0.76, 95% CI: 0.61-0.95)], to have died within 1 year [(OR=2.34, 95% CI: 1.30-4.24) and (OR=3.02, 95% CI: 1.71-5.41)], and have incurred higher costs [(OR=1.18, 95% CI: 1.07-1.31) and (OR=1.25, 95% CI: 1.13-1.38)] compared to diarrhoea control patients. Considering only patients infected by toxigenic strains, a toxin positive test was a predictor of only CDI severity (OR=3.18, 95% CI: 1.05-9.60). The addition of a third and confirmatory diagnostic test was cost-saving when considering the use of a high cost antibiotic.

When procalcitonin (PCT) was measured within 72 hours after the *C. difficile* test in cohort patients, high levels of PCT were associated with CDI diagnosis (OR=1.76, 95% CI: 1.04-2.58), CDI severity (OR=1.56, 95% CI: 1.18-2.07), long-term mortality (OR=1.43, 95% CI: 1.15-1.77) and with increased risk of delayed discharge (HR=0.87, 95% CI: 0.80-0.95). A toxin positive result was only predictive of time to discharge when PCT was one of the covariates of the models.

Cost-effective interventional measures identified by the systematic review undertaken in this thesis were screening all patients during admission, vaccination in a simulation model, treatment with fidaxomicin (FDX) and faecal microbiota transplantation (FMT) via colonoscopy.

Multivariable analysis showed that costs of hospitalisation were higher for CDI cases than diarrhoea control patients in phase I (£5,761 vs £4,924 for cohort group and £6,272 vs £5,151 for audit and cohort groups). During phase II, CDI cases treated with FDX (£6,355 and £5,694) and GDH+/TOX- patients treated with FDX (£5,746 and £5,448) were more expensive than diarrhoea control patients (£4,227 and £4,251).

In conclusion, this thesis has presented clinical and economic perspectives of CDI in epidemic and endemic phases in a secondary healthcare setting. CDI is associated with a number of adverse clinical outcomes, such as higher mortality rates, longer time to discharge and hospitalisation costs, which have been highlighted in this thesis. Tackling CDI requires a multifunctional approach, including prevention and control measures, and better treatment strategies to decrease the incidence rates and improve outcomes in infected patients in a cost-effective manner.

Chapter 1

Introduction

1.1 Aetiology

Clostridium difficile infection (CDI) is one of the most common causes of healthcare-associated infection (HCAI) worldwide. The organism is anaerobic, Gram-positive, spore-forming bacillus and producer of exotoxins (Kelly et al., 1994). A disruption to the normal bowel flora usually caused by broad-spectrum antibiotics, plus the production of toxins when spores are converted to vegetative forms, cause an inflammatory condition that can reach the colon and develop a pseudomembranous colitis (Kelly et al., 1994, Spencer, 1998).

1.2 Risk factors

Antibiotic exposure has been considered one of the major risk factors for the development of CDI and cephalosporins (CF), clindamycin (CLI) and fluoroquinolones (FQ) the most frequent antibiotics associated with the disease (Bartlett, 2010, Spencer, 1998). Besides, long-term hospitalisation and exposure to the bacteria, male gender, advanced age (more than 65 years), age less than 1 year with co-morbidity, prolonged duration of diarrhoea, serious underlying illness, weakened immune system and surgery on the digestive system are also risk factors for the disease (NHS, 2012, Goudarzi et al., 2014). Moreover, gastric acid suppressants have been related to increased risk of CDI as can decrease the protective effect of gastric acid and altering the microbiota but this is still unclear and controversial, as some studies have not shown association (Surawicz et al, 2013, Tariq et al, 2017). The findings of Novack et al study (Novack et al, 2014) have suggested a potential bias when recruiting control patients as a reason for the discordant results.

1.3 Pathogenesis

The spread of *Clostridium difficile* is via the oral-faecal route after oral ingestion of spores or vegetative forms of the pathogen. In the lower gastrointestinal tract (Figure 1.1), the bile acids and other substances induce spore germination and vegetative growth (Abt et al., 2016). Due to the disruption of gut microbiota, vegetative cells invade the large intestine, interacting and adhering to epithelial cells (Abt et al., 2016, Usacheva et al., 2016, Goudarzi et al., 2014). The consequent multiplication of the micro-organism and the production of toxins cause injuries to the cells and induce an inflammatory process in the mucosa by neutrophil infiltration (Usacheva et al., 2016, Abt et al., 2016).

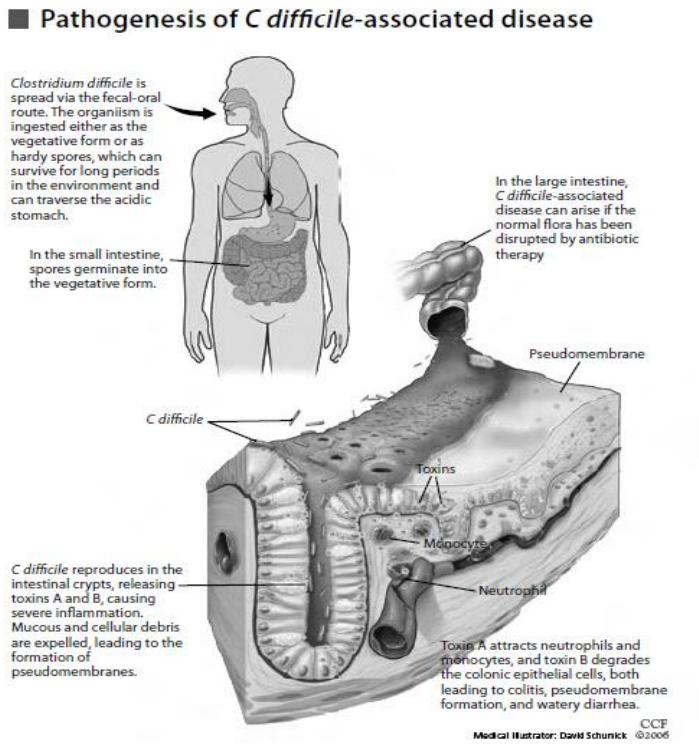

Figure 1.1 Pathogenesis of CDI (Source: Association for Professionals in Infection Control & Epidemiology, 2008)

The major toxins produced by *C. difficile* are the enterotoxic toxin A (tcdA) and the cytotoxic toxin B (tcdB). Studies have diverged about the role of the tcdA in the virulence process (Usacheva et al., 2016), as some authors suggested a synergistic effect between tcdA and tcdB (Kuehne et al., 2010, Kuehne et al., 2011) whilst other authors suggested that it has no relevance as only toxin B is cytotoxic (Carter et al., 2012). A binary toxin called *C. difficile* transferase (CDT) has also been identified in CDI case strains. Although the role of CDT is still not completely known, higher mortality rates and severe disease were associated with strains that express this toxin, such as the hypervirulent strain NAP1/BI1/027 (RT027) (Gerding et al., 2014, Depestel and Aronoff, 2013, Berry et al, 2017).

1.4 Epidemiology

CDI epidemiology has been changing during the years. According to a meta-analysis conducted in the United States (US) (Zimlichman et al., 2013), *C. difficile* was the healthcare-associated infection with the second highest number of cases per year (133,657) after surgical site infection (SSI) with 158,369 cases per year. In some US areas it is already considered the most common cause of HCAI (Depestel and Aronoff, 2013). Data from 2011 suggested more than 450,000 cases (147 cases/100,000 population) of those 65% were HCAI and led to 29,000 deaths in a year in the US (Lessa et al., 2015). In the same period, 19,000 cases (36 cases/100,000 population) were reported in the United Kingdom (UK) (PHE, 2017).

1.4.1 Outbreak

Last decade witnessed a significant upsurge in the incidence and severity of CDI in North America and Europe (Loo et al., 2005, Kuijper et al., 2006) with the spread of the new epidemic strain RT027. This epidemic strain type is widely acknowledged to be more virulent and associated with severe illness and increased mortality and recurrence rates (Deneve et al., 2009). Since 2003 outbreaks have been reported in the US, Canada and Europe. In the UK, the number of cases started rising in 2006-2007 when notification of all CDI cases from National Health Service (NHS) Trusts to Public Health England (PHE) became mandatory. For this reason, this outbreak put healthcare systems under severe strain, triggering major reviews in antimicrobial policies and introduction of stringent hygiene and cleanliness measures, often not practically sustainable, to try to control the number of infected patients and to avoid spending money unnecessarily (Department of Health & Health Protection Agency, 2008, Simor, 2010).

In England, surveillance of *C. difficile* started in 1990 as voluntary monitoring, becoming mandatory in people aged 65 years and over in 2004 and including people aged from 2 years and over in 2007. Figures with number of cases per financial year since 1990 and deaths related to CDI since 2001 in England from National Statistics (Office for National Statistics, 2013) and PHE (PHE, 2017) databases are presented in Figures 1.2 and 1.3.

The epidemic period started with the increasing in the number of CDI cases around 2002, reaching a peak with the emergence of the hypervirulent strain when 55,000 cases and 11,000 deaths whose certificates mentioned *C.*

difficile as a cause or underlying cause of death were reported in England (PHE, 2017, Office for National Statistics, 2013). The incidence of CDI decreased year by year falling by 70% in 2010 as a result of interventional measures implemented by the healthcare settings. In 2012 the number of cases have remained relatively stable consistent with an endemic period.

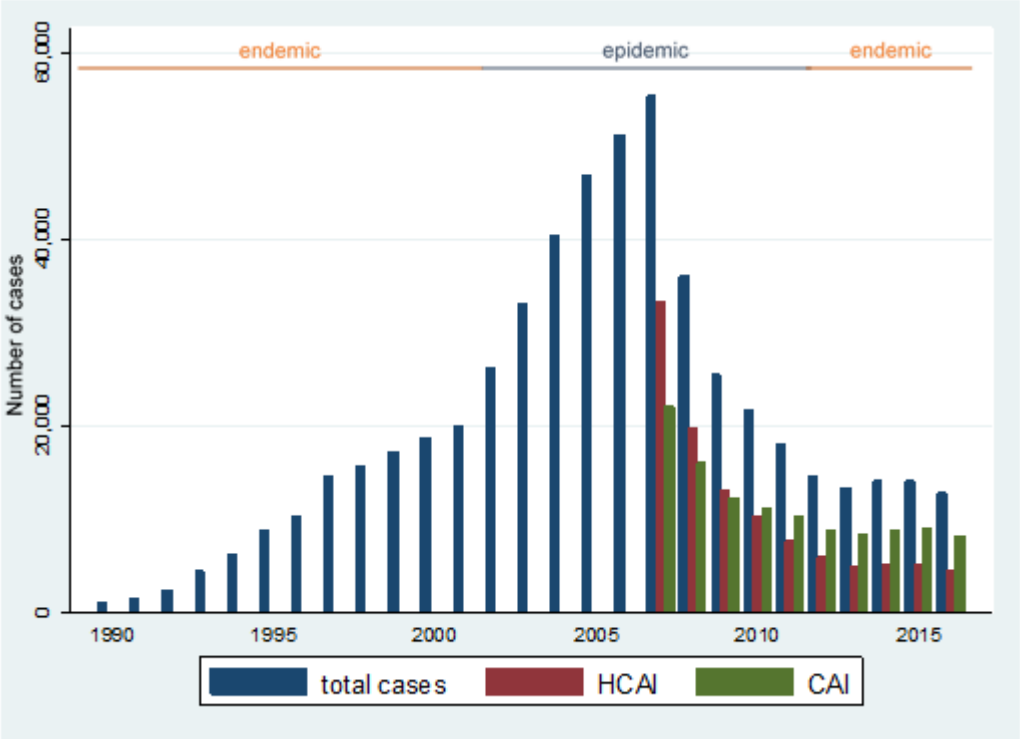

Figure 1.2 Number of HCAI and CAI CDI cases in England between 1990 and 2016 (Source: Public Health England, 2017)

PHE reports after 2007 presented both total CDI cases and cases by mode of acquisition the bacteria, whether if it is a HCAI or a community-acquired infection (CAI). A HCAI case is usually defined as an infection detected within 48h after the patient admission in a healthcare setting if the patient received healthcare treatment inside or outside the hospital 30 days before the infection, or was hospitalised for at least 2 days within 90 days before the

infection, or lived in a nursing home (Friedman et al, 2002; Cardoso et al, 2014). Figure 1.2 shows that the number of CDI cases considered HCAI has decrease during the years and in 2010 cases not related to healthcare interventions have become higher than HCAI cases in England. However, these data may not be accurate as the PHE reports all cases and Trust apportioned cases, which is considered when specimen was taken at an Acute Trust and after the fourth day of the hospital admission and patient was hospitalised, a day-patient or an emergency assessment patient (PHE, 2017). Also, a systematic review published in 2014 suggested that 2 or more days of hospitalisation in the previous year and treatment with broad spectrum antibiotics in the last month should also be considered to define a HCAI case (Cardoso et al, 2014).

Data were also reported by England regions (Figure 1.4, 1.5 and 1.6). Heat maps represent case rates/100,000 population (Figure 1.7) and death rates/1,000,000 population (Figure 1.8) between 2009 and 2016 and between 1999 and 2002 per England region. A higher incidence occurred in the North West and there was a higher rate of CDI cases in Northern regions whilst the London region showed the lowest rate during the whole period. Death related to *C. difficile* had a higher rate in the South West region before the outbreak, the Midlands regions during the outbreak and Northern regions after the outbreak.

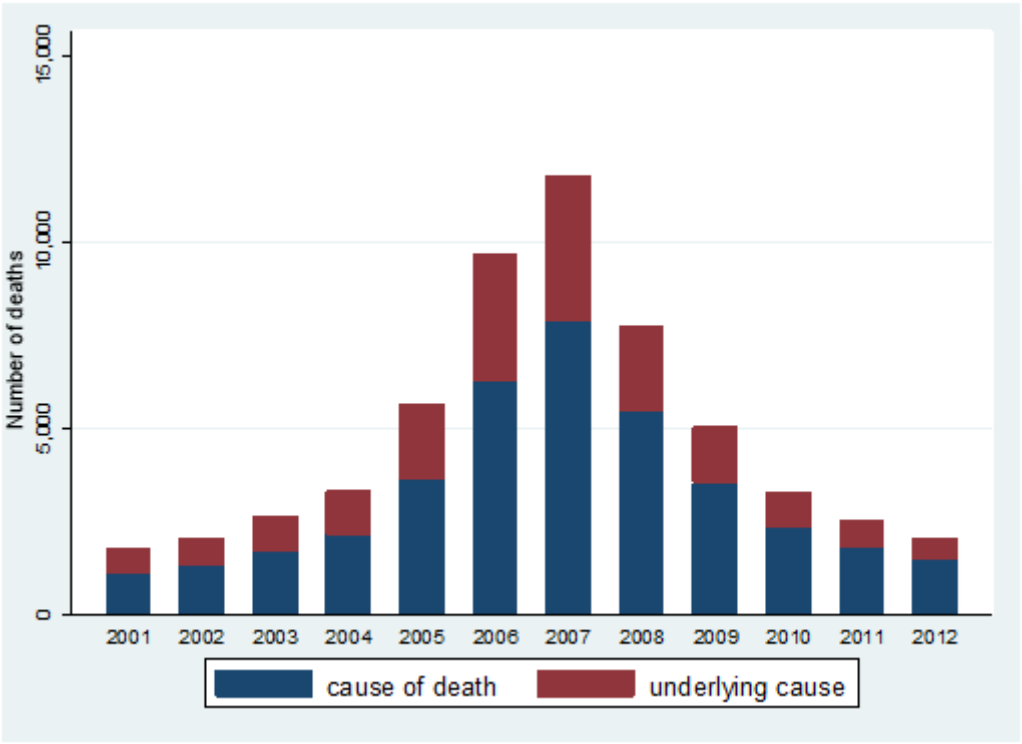

Figure 1.3 Number of deaths related to *C. difficile* in England between 2001 and 2012 (Source: Office for National Statistics, 2013)

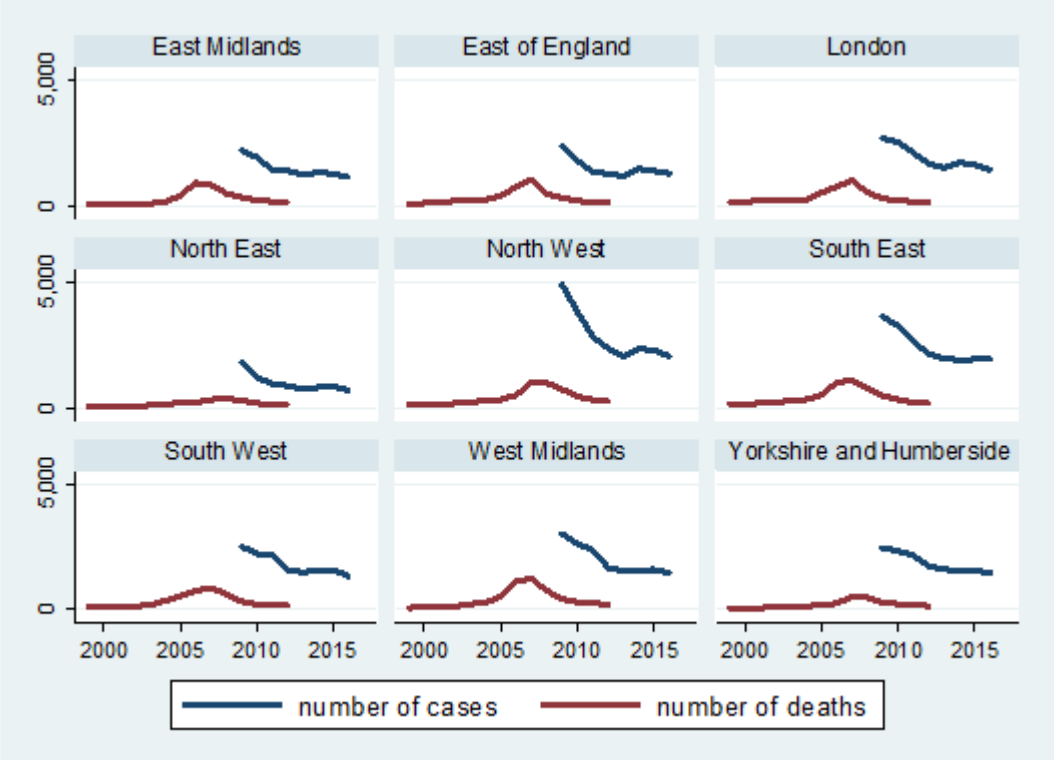

Figure 1.4 Number of CDI cases and CDI deaths between 1999 and 2016 by England regions (Source: Office for National Statistics, 2013 & Public Health England, 2017)

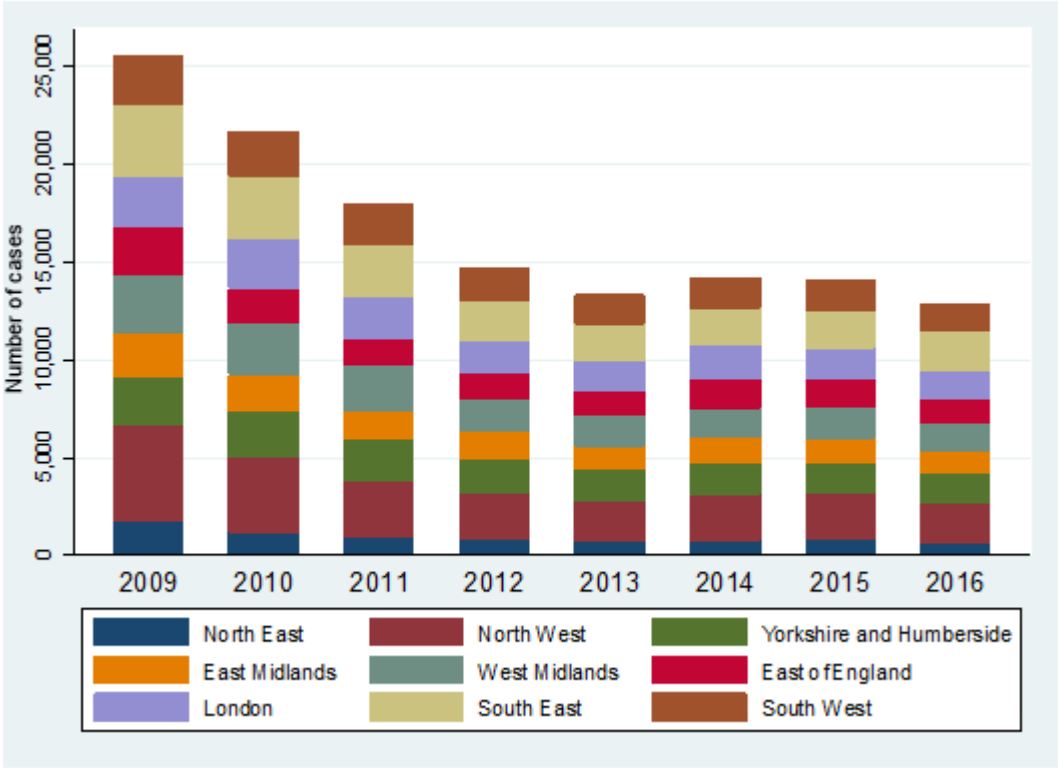

Figure 1.5 Number of CDI cases between 2009 and 2016 by England regions (Source: Public Health England, 2017)

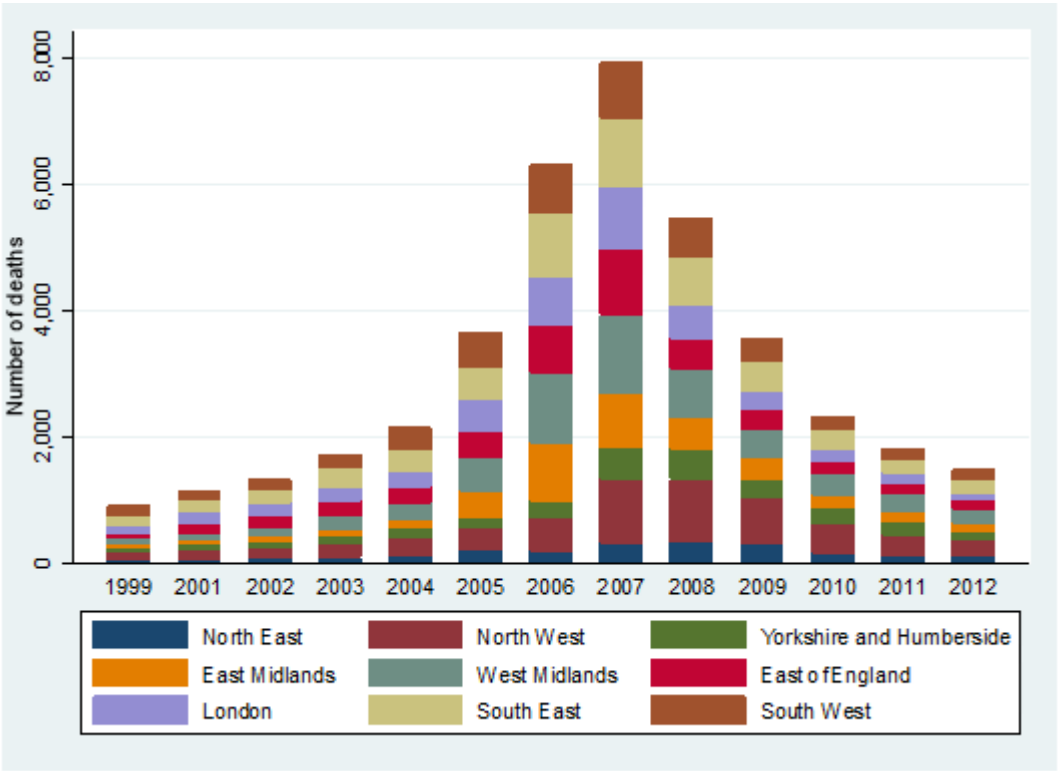

Figure 1.6 Number of deaths related to *C. difficile* between 1999 and 2012 by England regions (Source: Office for National Statistics, 2013)

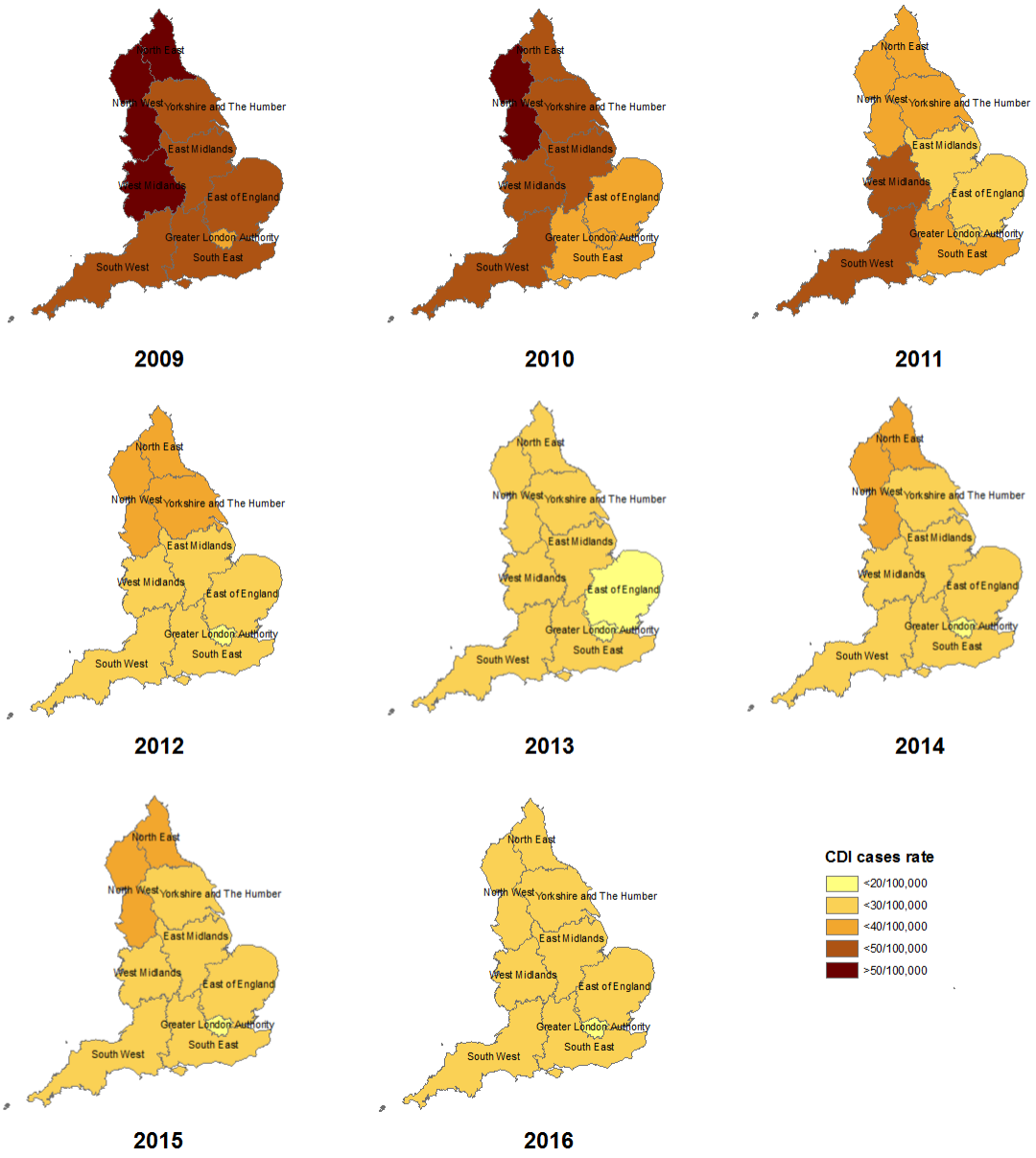

Figure 1.7 CDI cases rate in England between 2009 and 2016 (Source: Public Health England, 2017)

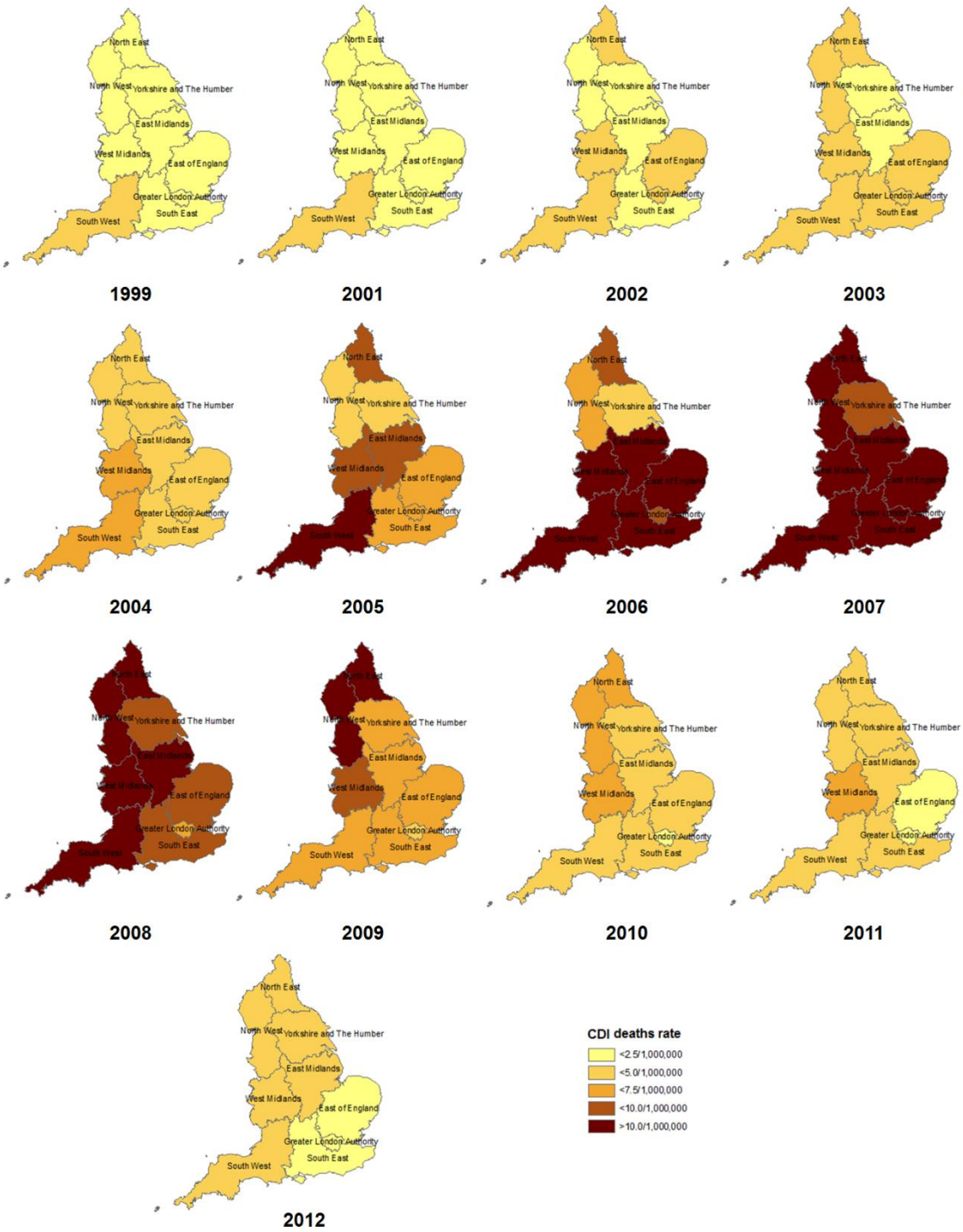

Figure 1.8 CDI death rate in England between 1999 and 2016 (Source: Office for National Statistics, 2013)

1.5 Transmission

Spores, the inactive state of the bacteria, are resistant to cleaning and disinfection measures and can survive in the environment for a long period of time (Fekety et al., 1981). Thereby, infected humans (symptomatic or asymptomatic) and inanimate objects are two major reservoirs of *C. difficile* and the transmission can occur from the healthcare environment or via patient care activities (APIC, 2008). The faecal-oral route can allow spores to contaminate bedclothes, bathroom fixtures, medical equipment and clothing, and then spread when healthcare staff or other people touch contaminated areas or infected patients. Studies have also reported potential airborne transmission, as bacteria could be isolated from the air of patients' rooms (Roberts et al., 2008, Best et al., 2010), however clinical relevance is limited (Donskey, 2010) as the number of spores recovered was low. Spores were also recovered from the air of a pig farm (Keessen et al., 2011) and toilet area, including floor, cistern and toilet seat (Best et al., 2012).

Recent advances in the molecular profiling of CDI using next generation sequencing are likely to improve the understanding of *C. difficile* epidemiology, as well as its emergence, spread and transmission in both hospitals (microevolution) and worldwide (global evolution) (He et al., 2012).

1.6 Signs and symptoms

Clinical presentation varies from asymptomatic carriage to death, including a wide spectrum of manifestations such as diarrhoea, antibiotic-associated colitis without pseudomembrane formation and fulminant colitis. These

manifestations are easily confounded with other intestinal diseases making the diagnosis more difficult. The most common symptom presented in around 90% of the patients is brown or clear watery diarrhoea (Knoop et al., 1993). Asymptomatic carriers are not frequent among healthy adults ranging from 0 to 15%, in contrast with healthy neonates and infants whose rate varies from 18 to 90% (Furuya-Kanamori et al., 2015).

PHE published a guideline assessing the severity of CDI considering clinical manifestation as Bristol Stool Chart, levels of white cell count (WCC), levels of serum creatinine, temperature, blood pressure, evidence of colitis and ileus or toxic megacolon (PHE, 2013), as showed in Table 1.1. Thus, CDI can be categorised as mild, moderate, severe or life-threatening.

Table 1.1 CDI severity grading

Severity	Bristol stool chart/day	WCC	Serum creatinine	Other manifestation
Mild	<3 type 5-7	.	.	.
Moderate	3-5	<15 x10^9/L	.	.
Severe	variable	>15 x10^9/L	>50% increase above baseline	Temperature >38.5°C Evidence of severe colitis
Life-threatening	variable	>15 x10^9/L	>50% increase above baseline	Temperature >38.5°C Evidence of severe colitis Hypotension Partial or complete ileus or toxic megacolon CT evidence

WCC: white cell count; CT: computed tomography.

1.7 Diagnosis

Diagnosis is based on clinical manifestations and on identification of the toxigenic organisms or detection of toxins. It is strongly recommended that only symptomatic patients should be tested for *C. difficile*, unless for epidemiological purposes (Cohen et al., 2010, Surawicz et al., 2013). The best diagnostic test has not been established yet but the recommendation is a combination of tests as this can produce reliable results. According to the Public Health England UK (PHE, 2012), the NHS has three main options of tests to detect CDI: toxin (TOX) enzyme immunoassays (EIAs), nucleic acid amplification test (NAAT) and glutamate dehydrogenase (GDH) EIA, but the first option should be NAAT or GDH EIA and the other a TOX EIA test (Department of Health, 2012a). In US healthcare settings, the use of a 2-step or 3-step method consisting of GDH EIA as initial screening followed by TOX EIA or NAAT or NAAT to confirm discordant EIA result is recommended. However toxin assays have been switched to NAAT for detection of toxigenic *C. difficile* and it has been performed as a stand-alone test (Cohen et al., 2010b, Surawicz et al., 2013, Fang et al., 2017). Sensitivity, specificity and costs of the different tests must be checked when choosing the best test to diagnose CDI (Table 1.2).

Table 1.2 Comparison of diagnostic tests for C. difficile

	Diagnostic Test	Sensitivity	Specificity	Turnaround time	Costs	Availability
Toxin detection	Cytotoxin assay	+++	+++	48 hours	$15-25	Limited
	Enzyme Immunoassay (EIA)	+	+++	< 24 hours	$5-15	Widely
	NAAT	+++	+++	< 1 hour	$20-50	Widely
Organism detection	Common antigen testing (GDH antigen)	+++	+	15-45 minutes	$5-15	Widely
	Stool culture	+++	+	72 hours	$5-10	Limited

(Source: Surawicz, 2013)

Toxin assay can detect both toxins A and B but its sensitivity is low and specificity is high. The production of the enzyme GDH by *C. difficile* can be detected by an EIA assay with high sensitivity but low specificity as all strains, both toxigenic and non-toxigenic, produce the enzyme at high levels. A combined test that detects GDH and toxin in one assay was developed and became available on the market. NAAT which uses the polymerase chain reaction (PCR) technology has a high negative predictive value (NPV) and its sensitivity was reported to be 88-100% (Burnham and Carroll, 2013). NAAT does not detect the *C. difficile* toxin but the gene of organisms that can produce toxins and has higher costs compared to others. However it is a rapid test and overcomes one of the main limitations of GDH screening, which lacks specificity for toxigenic strains (Burnham and Carroll, 2013, Polage et al., 2015). Stool culture is important for epidemiological studies but it is not clinically useful, as its sensitivity is low and results take three days to be ready (Cohen et al., 2010).

1.8 Treatment

The current standard treatment for CDI (Table 1.3) is metronidazole (MTZ) or vancomycin (VAN). In 2011/2012 oral fidaxomicin (FDX) was also approved for this purpose in the US and Europe. VAN was considered superior to MTZ for the treatment of severe cases in a clinical trial (Johnson, 2014) but it was inferior to FDX in recurrent cases when using whole-genome sequencing (Eyre, 2014). Therefore, the choice of treatment is usually clinical-based and according to the severity of the disease. PHE (PHE, 2012), Society for

Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) (Cohen et al., 2010) and American College of Gastroenterology (Surawicz et al., 2013) recommend oral MTZ 400 to 500 mg three times a day (tds) for 10 to 14 days for mild and moderate CDI. The treatment of severe CDI should be oral VAN 125 mg four times a day (qds) for 10 to 14 days and in cases of high risk of recurrence and in the elderly with multiple comorbidities and in treatment with antibiotics, FDX 200 mg twice daily (bd) should be considered. Oral VAN up to 500mg qds for 10 to 14 days and MTZ 500mg tds is recommended for life-threatening CDI. Oral FDX 200mg bd is also indicated for recurrent cases. Intravenous treatment or via enema are also options to be considered when there are restrictions or complicated CDI. The most updated algorithm recommended in the UK is presented in the Figure 1.9.

Table 1.3 Treatment for CDI

Antibiotic therapy	Regimen	Cost/10 days ($)	Cost/10 days (£)
Metronidazole	500 mg tds	$22.00	£2.53
Vancomycin	125 mg qds	$680.00	£188.27
Fidaxomicin	200 mg bd	$2,800.00	£1,350.00

(Source: Surawicz et al, 2013, NICE, 2012)

Faecal microbiota transplant (FMT) has become more popular as evidences have suggested potential benefits for treatment of recurrent, severe and complicated cases (Kassam et al., 2013, Dodin and Katz, 2014). The number of published studies has showed a three-fold increase in the last 3 years. The

procedure aims to replace good bacteria by transplanting faecal matter from a healthy donor via colonoscopy, endoscopy, sigmoidoscopy or enema. National Institute for Health and Care Excellence (NICE) recommends the use of FMT only for recurrent cases when there is failure to respond to standard treatment, according to evidence of efficacy and safety of published studies (NICE, 2014). In the guidelines of the American College of Gastroenterology FMT is recommended in the third recurrence (Surawicz et al., 2013). A recent systematic review with 37 (Quraishi et al., 2017), 23 (van Beurden et al., 2017) and 35 (Drekonja et al., 2015) studies showed efficacy of FMT treatment for recurrent, severe and complicated cases and insufficient evidence for initial or refractory cases, respectively.

Moreover, clinical trials have been conducted to study the potential efficacy and safety of novel treatment for CDI such as cadazolid, ridinilazole and bezlotoxumab. In the phase 2 study, cadazolid demonstrated efficacy and safety (Louie et al., 2015); ridinilazole showed a clinical cure rate in 10 days higher than VAN and no recurrence after 30 days of treatment (Vickers et al., 2017); bezlotoxumab IV, when administered with standard therapy, showed lower recurrence rate compared to a placebo in a phase 3 trial (Wilcox et al., 2017).

There are other alternative options for CDI treatment or prevention, such as probiotics, anion exchange resin, non-toxigenic *C. difficile* (NTCD), fusidic acid, rifampicin and rifaximin, but they are not commonly used nowadays. There is no significant evidence to support the use of probiotics (Cohen et al., 2010), anion exchange resin is not recommended for this purpose (PHE, 2012), NTCD is not licensed but could be used as a supplement to the

standard therapy preventing relapses (Musher and Koo, 2016; Gerding et at, 2015), fusidic acid role is unclear but the resistance rate is high (PHE, 2012), rifampicin had no clinical trial reported (PHE, 2012) and rifaximin has been used for refractory cases but it is not approved for the treatment of CDI (Al-Jashaami and DuPont, 2016).

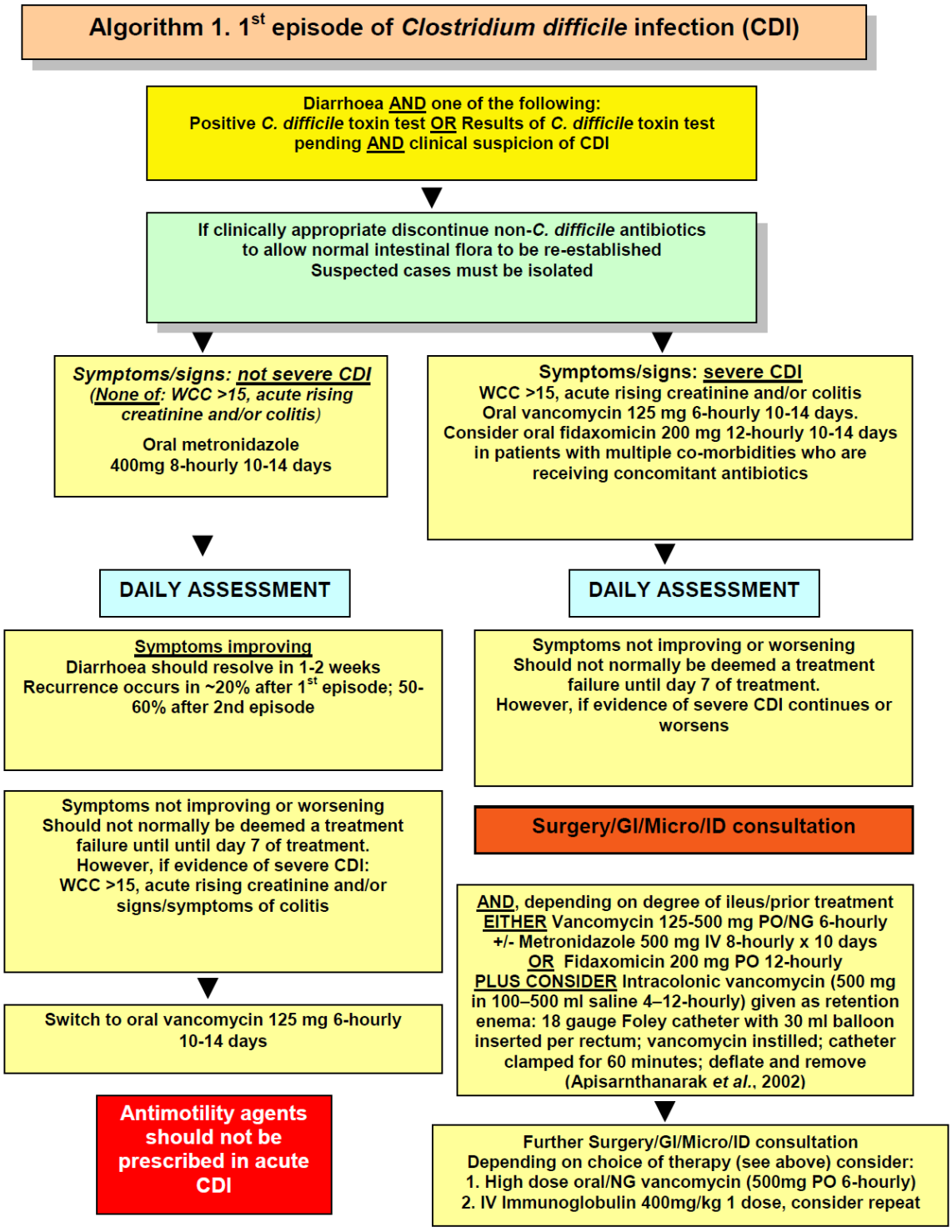

Figure 1.9 Algorithm for management of first episode of CDI (Source: Public Health England, 2013).

1.8.1 Recurrence

Recurrent episodes can affect 15 to 30% of patients after the first infection and this number is higher in subsequent recurrences (Eyre et al., 2012, Johnson, 2009). Recurrence can be due to either relapse when there is an infection with the same strain or reinfection when the new infection is caused by a different strain within the period of 90 days after the first infection. Inadequate antitoxin antibody response, severity of initial infection, persistent disruption of the colonic flora, advanced age, continuation of non-*C. difficile* antimicrobial therapy following a first episode of CDI, long hospital stays and concomitant receipt of antacid medication are some of the risk factors that can contribute to the development of recurrent episodes (Johnson, 2009). A recent meta-analysis published (Tariq et al., 2017) suggests an association between the use of gastric acid suppressant and the risk of recurrences after revision of 16 studies. This condition is a challenge for the treatment and can persist for months or years (Johnson, 2009, Eyre et al., 2012). Thus, it is recommended and important to identify the risk factors and treat the patient correctly (Figure 1.10) to try to avoid potential recurrences.

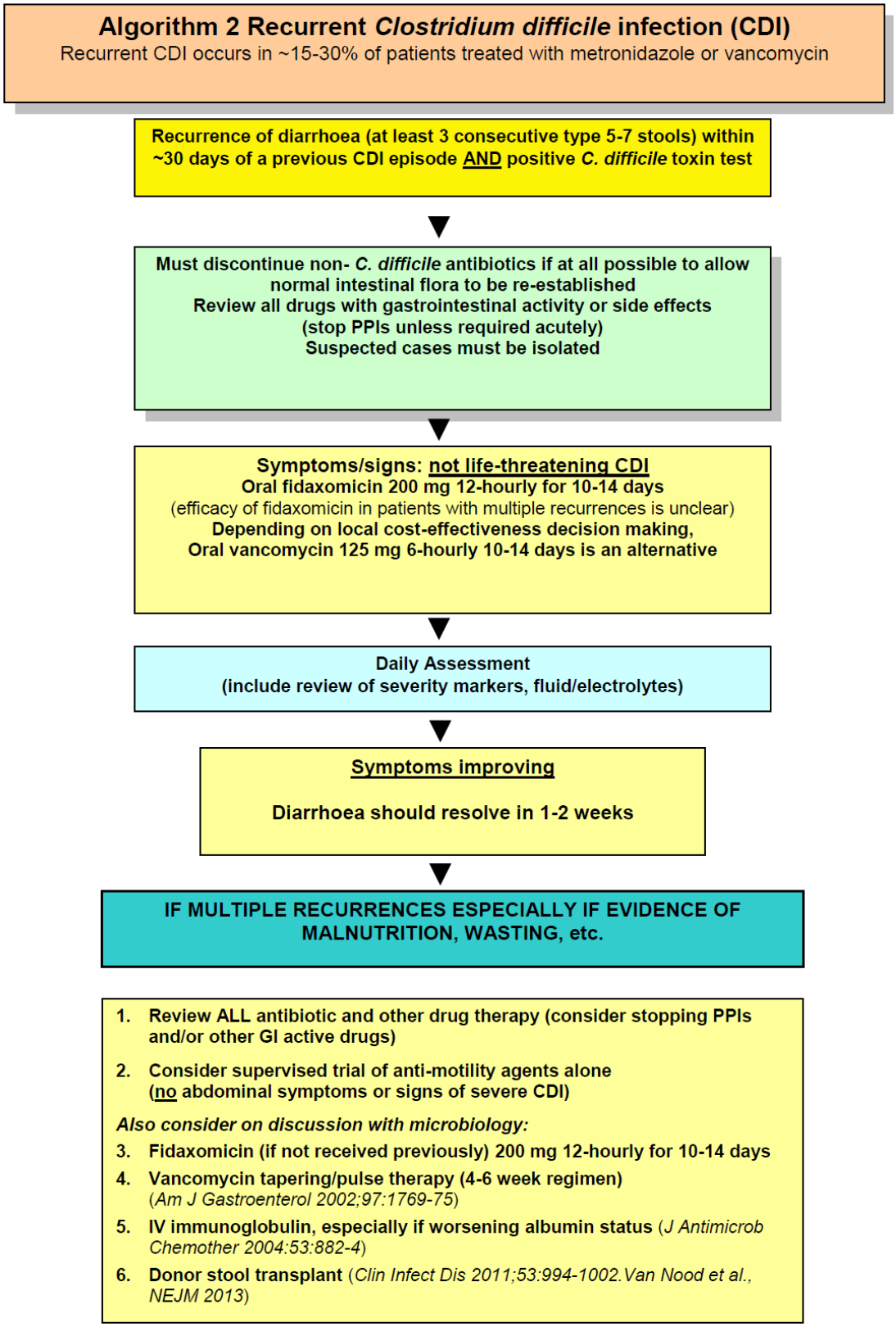

Figure 1.10 Algorithm for recurrence of CDI (Source: Public Health England, 2013).

1.8.2 Resistance

C. difficile can also resist the majority of routine antimicrobials, such as ampicillin, amoxicillin, CF and CLI (APIC, 2008). Some antibiotics are also known to promote CDI and this has changed during the years; CLI showed a high risk in the 70s, CFs around the 80s and 90s and fluoroquinolones in the 2000s (Spigaglia, 2016). Resistance is multifactorial and influenced mainly by local and national policies and prescribers, thus resistance rates are variable between countries, cities and hospitals. Considering 30 studies between 2012 and 2015, ciprofloxacin (CIP), a second-generation FQ, showed resistance in around 99% of strain tested and cefotetan (CTT) and cefoxitin (FOX), second-generation CFs, in 79%. Resistance rates of CLI, CFs, erythromycin (ERY) and FQs were on average 55%, 51%, 47% and 47%, respectively (Spigaglia, 2016).

Some strains also showed resistance to the antibiotics for treatment of CDI, but resistance rates are usually low. In a review, MTZ showed in general 0-0.11% resistance and VAN 0-1.2%. However, the rates in Iran and Israel were higher with 5.3% and 18% for MTZ and 8% and 47% for VAN (Spigaglia, 2016). In Israel, this reduced susceptibility to antibiotics in 2014 was related to the dissemination of R027 as 65 patients presented on average 87.7% and 44.6% resistance rate for MTZ and VAN (Adler et al., 2015). The indiscriminate use of MTZ in Iran could be the cause of increased resistance in the study conducted in 2010/2011 (Goudarzi et al., 2013). Reduced susceptibility of FDX was rarely reported (Spigaglia, 2016) and no evidence was found in a study (Freeman et al., 2015) and a review published in 2016 (Tang et al., 2016).

1.9 Prevention and control

Prevention and control measures were implemented to avoid direct and indirect contamination and to reduce the number of infected patients in the healthcare sites. The Health Protection Agency (HPA) has published a 3 grade system (Table 1.4) with recommendations to manage CDI: a mnemonic protocol called SIGHT (Table 1.5) should be used by doctors and healthcare staff in suspected cases. The Association for Professionals in Infection Control & Epidemiology (APIC) (Table 1.6) also suggests contact precautions, hand hygiene and environmental control as the three main approaches to deal with the bacteria (APIC, 2008).

The components of contact precautions are: (a) patient placement (private room and bathroom and when it is not possible, a dedicated *C. difficile* ward for isolation); (b) personal protective equipment (PPE, such as gloves and gowns); (c) patient transport (infected patient transportation should be limited and hand hygiene should be performed by patients and PPE should be used and discarded by healthcare professionals); (e) patient care equipment, instruments, devices and the environment (as *C. difficile* can contaminate all of the environment and equipment and can persist for months, all healthcare sites should have cleaning and disinfection plans); (e) discontinuing contact precautions (after the end of the symptoms, contact precautions may be discontinued); and (f) assessment of adherence to isolation precautions (Department of Health & Health Protection Agency, 2008, APIC, 2008).

Table 1.4 Grade system for CDI management

Grade	Strength of evidence
A	Strongly recommended and supported by systematic review of randomised controlled trials (RCTs) or individual RCTs.
B	Strongly recommended and supported by non-RCT studies and/or by clinical governance reports and/or the Code.
C	Recommended and supported by group consensus and/or strong theoretical rationale.

(Source: Health Protection Agency, 2013)

Table 1.5 SIGHT protocol for CDI management

S	**Suspect that a case may be infective where there is no clear alternative cause for diarrhoea**	**B**
I	Isolate the patient and consult with the infection control team (ICT) while determining the diarrhoea	B
G	Gloves and aprons must be used for all contacts with the patient and their environment	B
H	Hand washing with soap and water should be carried out before and after each contact with the patient and the patient's environment	A
T	Test the stool for toxin, by sending a specimen immediately.	B

(Source: Health Protection Agency, 2013)

Besides gloves, hand washing with a non-antimicrobial or an antimicrobial soap and water is also very important to control the spread of the bacteria. Not only must healthcare professionals clean their hands properly, but also patients and their family must do it. For this purpose, it is recommended to teach hand hygiene and bathing to all patients and their families and promote understanding of the infection, spread of bacteria, how to reduce the spread of the disease, the risks of acquiring it and how to clean their homes (APIC, 2008, Department of Health & Health Protection Agency, 2008). Hand washing is known to be one of the most important measures in control and prevention of infections.

Table 1.6 Control measures and grade of recommendation

Variable	Strength of recommendation	Reference(s)
Hand hygiene	A-II	
Contact precautions		
Glove use	A-I	Johnson et al[150]
Gowns	B-III	
Use of private rooms or cohorting	C-III	
Environmental cleaning, disinfection, or use of disposables		
Disinfection of patient rooms and environmental surfaces	B-II	
Disinfection of equipment between uses for patients	C-III	Brooks et al[79]
Elimination of use of rectal thermometers	B-II	Mayfield et al,[76] Wilcox et al[78]
Use of hypochlorite (1,000 ppm available chlorine) for disinfection	B-II	

(Source: Cohen, 2010)

The entire environment, including surfaces and objects, must be cleaned daily using specific environmental disinfectants that are also able to kill the vegetative form and spores. The HPA recommends chlorine-containing cleaning agents (at least 1,000 ppm of available chlorine) (Department of Health & Health Protection Agency, 2008). Vaporised hydrogen peroxide can

also be used to clean and disinfect the environment in private rooms and isolation wards. Healthcare sites should provide a checklist to help staff to confirm that all areas and objects were cleaned and disinfected and also provide a team to monitor the cleaning process routinely. Besides, meetings with the infection control team, cleaning staff and healthcare professionals should be held to discuss this subject (Department of Health & Health Protection Agency, 2008, APIC, 2008).

Antibiotic stewardship is considered the most useful measure to control the bacteria and this has been widely implemented to reduce the inappropriate use of antibiotics. Between 1997 and 2013, CFs and FQs were the classes of antibiotics with more restrictions according to a meta-analysis that included 16 studies (Feazel et al., 2014). This study also concluded that control of these classes was effective in decreasing the incidence of CDI. A genomic study has shown a decrease in FQ-resistant isolates after restriction of use of fluoroquinolones (Dingle et al., 2017). In a hospital in the UK, interventions have been implemented since 2003 aiming to decrease the number of HCAI and included: antibiotic stewardship, surveillance and feedback, infection control standards and practice, education and training, governance framework/programmes and leadership and a national policy and campaign (Marufu et al., 2015). Restriction of quinolones and CFs use between 2007 and 2011 was associated with reduction of CDI incidence in accordance with other studies (Marufu et al., 2015). An American hospital showed a decrease in the number of cases, total expenditure and resistance rate after the implementation of CLI restriction. Resistance rate dropped from 91% to 39% in 26 months (Climo et al., 1998).

However, one decade after the outbreak, HCAI CDI has been decreasing over the years and asymptomatic and untested symptomatic patients have been suggested to be a source of *C. difficile* and that isolation of confirmed patients may not be sufficient to reduce the transmission of disease. Moreover, *C. difficile* was recovered from environmental sources such as piglets, cattle, horses and poultry, water, soil and household environs (Martin et al., 2016).

1.10 Policy

1.10.1 Costs and Health economics

CDI causes a substantial economic burden for the healthcare systems and adds an extra layer of complexity to the patient's management due to its easy spread and resilience to environmental control measures. It is estimated that CDI extends hospital stays by 1 to 3 weeks (Chang et al., 2007, Forster et al., 2012) and that in-patients are over twice as likely to die of CDI than methicillin-resistant *Staphylococcus aureus* (MRSA) in England and Wales (Department of Health & Health Protection Agency, 2008).

A systematic review of CDI in some European countries showed that the median length of stay (LoS) ranged from 7.8 to 48.8 days (Wiegand et al., 2012), while in the US from 6.6 to 18 days (Ghantoji et al., 2010). The attributable length of stay in the US was between 2.8 and 5.5 days (Dubberke and Olsen, 2012). The HPA reported that approximately 1 in 4 patients has recurrent episodes and that the majority are subsequently re-admitted to hospital, vastly increasing direct and indirect costs (Department of Health & Health Protection Agency, 2008). Hospital stay is considered not only a risk

factor to the development of CDI, but also is directly related to increasing costs, comprising about 85-94% of total costs (Kyne et al., 2002, Wiegand et al., 2012). An extra length of stay can amount on average to €14,000 per patient with a bed day in an intensive care unit being set at €1,200, almost twice as high as in a regular ward (Magalini et al., 2012).

A review by Ghantoji (Ghantoji et al., 2010) estimated that total costs for CDI patients was between $9,822-13,854, over 40% more when compared to the matched control group ($6,950-9,008). It has been suggested that the cost per episode is in the range of €5,000-15,000 (Kuijper et al., 2006). In addition, estimates from three health-economic studies in the US suggest that the overall management costs associated with *C. difficile* range from $436 million to more than $3 billion (Kuijper et al., 2006, Simor, 2010, Deneve et al., 2009). Recent reviews suggested that the direct and indirect burden to the healthcare systems are projected to be between $1.0-4.8 billion for the US (Dubberke and Olsen, 2012) and around €3 billion for the European Union (Kuijper et al., 2006, Wiegand et al., 2012).

Traditional economic studies on *C. difficile* usually rely on the collection of batches of data and are based on basic parameters such as hospitalisation times, costs of laboratory tests and associated antimicrobial therapies. In most studies indirect costs, the costs from days lost due to absence or productivity losses, are usually not calculated and these studies did not take into account outpatient costs, thus the overall costs could be underestimated (Ghantoji et al., 2010). Therefore, while cost analysis is useful for assessing direct costs, it poses difficulties for accurate ascertainment of indirect costs and cost-effectiveness of interventional measures, which require a robust framework for

the integration of information, often linked to individual patients' episodes, as well as a deep understanding of the disease epidemiology and dynamics. One of the main challenges for economic studies is the accurate measurement of disease recurrence since it is an inconstant clinical feature and difficult to predict and investigate.

1.10.2 Guidelines

Several guidelines have been published over the years in the US, Europe and the UK, mainly focused on prevention, treatment and diagnosis to help healthcare sites and professionals deal with CDI patients and CDI outbreaks. Some of them are listed below:

- *Clostridium difficile* infection: how to deal with the problem (Public Health England, 2008);
- Guide to the elimination of *Clostridium difficile* in healthcare settings (Association for Professionals in Infection Control & Epidemiology, 2008);
- *Clostridium difficile*: what it is, how to prevent, how to treat (Public Health England, 2009);
- *Clostridium difficile* infection (CDI): management in care homes (Public Health England, 2010);
- Clinical practice guidelines for *Clostridium difficile* infection in adults (Society for Healthcare Epidemiology of America and the Infectious Diseases Society of America, 2010);

- Treating *Clostridium difficile* infection with faecal microbiota transplantation (American Gastroenterological Association, 2011);
- *Clostridium difficile*: updated guidance on diagnosis and reporting (Public Health England, 2012);
- *Clostridium difficile* infection: fidaxomicin (National Institute for Health and Care Excellence, 2012);
- *Clostridium difficile* infection: guidance on management and treatment (Public Health England, 2013);
- Guidelines for Diagnosis, Treatment, and Prevention of *Clostridium difficile* Infections (American College of Gastroenterology, 2013);
- Faecal microbiota transplant for recurrent *Clostridium difficile* infection (National Institute for Health and Care Excellence, 2014);
- Update of the treatment guidance document for *Clostridium difficile* infection (European Society of Clinical Microbiology and Infectious Diseases, 2014);
- Infection prevention and control (National Institute for Health and Care Excellence, 2014);
- Guidance on Prevention and Control of *Clostridium difficile* Infection (CDI) in Care Settings in Scotland (Health Protection Network, 2014);
- WSES guidelines for management of *Clostridium difficile* infection in surgical patients (World Society of Emergency Surgery, 2015);

1.11 Aims

Clinical, epidemiological and molecular advances have showed the importance of trying to avoid new cases and recurrences. These advances have not been followed by systematic economic studies evaluating the recent impact of the disease on healthcare systems and the cost-effectiveness of major interventional measures. Moreover, the actual economic impact caused by several epidemic seasons of *C. difficile* on the NHS and healthcare systems has yet to be determined in more details. The situation clearly denotes a need for further economic and prevention studies.

Thus, we propose an investigation to:

1. Describe characteristics of CDI patients and diarrhoea control patients recruited in two epidemiological seasons and assess representativeness of those patients compared to patients who have not met the inclusion criteria;

2. Investigate the contribution of patients with discordant assay results as a reservoir of *Clostridium difficile* and potential cost-saving with the implementation of a confirmatory test;

3. Assess the use of procalcitonin (PCT) as a potential prognosis test for hospitalised patients infected by *Clostridium difficile*;

4. Conduct a systematic review on the economics of interventional measures for CDI;

5. Estimate the costs associated with CDI during the epidemic and endemic seasons in a large hospital setting.

Chapter 2

Clostridium difficile infection in Liverpool from 2008 to 2016

2.1 Introduction

CDI is one of the most common causes of hospital-acquired diarrhoea worldwide but there is patient and microbiological heterogeneity. *Clostridium difficile* ribotyping network report (PHE, 2014) has shown that England is becoming more heterogeneous with respect to *C. difficile* strains. Therefore, recruitment and characterisation of patients are important to understand this variability.

Biobanks have been developed as a health resource to help and improve diagnosis, prevention and treatment of different diseases (UK Biobank, 2017). Databases and biobanks can recruit a large number of individuals, being able to be representative of the general population. Also, they have been set up in many countries and are available in every continent. In the UK, the UK Biobank has recruited around 500,000 patients between 2006 and 2010 (UK Biobank, 2017) and The Infectious Diseases Biobank (IDB) has collected samples from patients infected with human immunodeficiency virus (HIV), hepatitis B and C and MRSA (IDB, 2017).

For CDI, some studies with databases and biobanks have been published in the literature. 45,341 patients from OptumInsight Clinformatics Database developed CDI and were recruited between 2001 and 2012 in the US (Ma et al., 2017), it contains information on insured patients from different regions of the country. In 2010/2011, 1,026 CDI cases were recruited from 47 facilities in Japan (Takahashi et al., 2014). A French study used the French national health insurance database which have more than 600,000 registered individuals to select 482 patients with a CDI diagnosis between 2007 and 2014

(Barbut et al., 2017a). In the UK, the *Clostridium difficile* Ribotyping Network for England and Northern Ireland has developed a tool to help hospitals to investigate and manage the disease with a molecular epidemiological service (PHE, 2016).

To understand clinical, economic and molecular aspects of this disease, patients infected with *C. difficile* were recruited at the Royal Liverpool and Broadgreen University Hospitals Trust (RLBUHT) in epidemic (2008-2012) and endemic (2012-2016) phases. Thus, the aim of this chapter was to present the cohort groups recruited and assess the representativeness of the cohorts compared to all patients tested for *C. difficile* in the setting during the same period.

2.2 Methods

2.2.1 Patient recruitment

2.2.1.1 Phase I

Recruitment occurred from July 2008 to March 2012. The cohort was composed of inpatients aged≥18 years, who had developed antibiotic-associated diarrhoea and had a sample tested for *C. difficile*. A stool positive for TOX, a positive clinical diagnosis and a positive bacterial culture were the inclusion criteria for CDI cases. Control patients were included in this study when they had antibiotic-associated diarrhoea but had negative tests for both stool TOX and bacterial culture. Thus, phase I patients were categorised as TOX+ or TOX-. This study was approved by the Liverpool Research Ethics

Committee (reference number 08/H1005/32). Informed consent form for both phases is presented in the appendix 1.

2.2.1.2 Phase II

Recruitment of phase II was similar to phase I, again, patients who had been tested for *C. difficile* and aged≥18 years were recruited from February 2013 to July 2015. In May 2012, a two-stage diagnostic algorithm for CDI – consisting of a glutamate dehydrogenase (GDH) enzyme immunoassay (EIA) and an EIA detecting *C. difficile* toxins A and B (TOX) – was implemented at the RLBUHT, the main recruiting site. Thus, the inclusion criteria for CDI cases were a stool specimen that was positive for TOX and GDH, a positive clinical diagnosis and a positive bacterial culture. Control patients were included when they had antibiotic-associated diarrhoea plus both negative test for stool TOX regardless of the GDH result and negative bacterial culture. In addition, a random sample of patients with inflammatory bowel disease (IBD) and healthy patients who were not tested for *C. difficile* were recruited. Based on these two diagnostic tests, three groups of patients were recruited during phase II:

- Patients categorised as cases (GDH+/TOX+),
- Potential *C. difficile* carriers (GDH+/TOX-),
- Patients categorised as controls (GDH-/TOX-).

2.2.1.3 Clinical audit

To fill the gap between the recruitment of the two cohorts and to increase the number of observations, a clinical audit titled “An effectiveness assessment of the on-going *Clostridium difficile* infection programme, overseen by Infectious Diseases, to audit/reaudit different strands facilitating quality improvement” was submitted in August 2015. Thus, it was possible to obtain information on patients tested between July 2008 and January 2016. The first phase (July 2008 to April 2012) included all patients tested before the introduction of the GDH detection and the second phase (May 2012 to January 2016) included patients tested for both TOX and GDH.

A list with all faecal samples sent to Liverpool Clinical Laboratories (LCL) was used to select the patients. Sample selection was according to test results and followed the priority: GDH+/TOX+, GDH+/TOX- and GDH-/TOX-. All GDH+/TOX+ samples were selected as the index episode and all samples from these patients retested within 90 days were excluded regardless of the result. The process was repeated for GDH+/TOX- samples and lastly for GDH-/TOX- samples. Duplicate samples were also excluded. Samples already included in both cohorts were excluded from the audit, however, the same patient could be included in more than one group (cohort or audit, phase I or phase II) when retested after 90 days.

Demographic and hospitalisation data were obtained without patients’ consent from the finance department when data were available. For samples tested after the implementation of GDH, clinical data on GDH+/TOX+ patients, GDH+/TOX- patients and a random sample of GDH-/TOX- patients were

collected using the databases described in the session 2.2.3. This audit was approved by Effectiveness Team at the RLBUHT (reference number AC03389).

2.2.2 Microbiological assessment

During phase I, a TOX A/B ELISA kit was used to test faecal samples for *C. difficile* toxin (Techlab, Blacksburg, USA). Culture was performed using Brazier's cefoxitin-cycloserine egg yolk agar (Lab M Ltd, Bury, UK) and incubated in an anaerobic chamber at 37ºC for 48 hours. Potential isolates were identified based on the characteristic smell, colonial morphology and fluorescence under long wave UV light. A latex agglutination test for *C. difficile* somatic antigen (Oxoid, Basingstoke, UK) was used to confirm the identification and purity of isolates that were stored on PROTECT beads at -70ºC (Technical Services Consultants Ltd, Heywood, UK). Standards methods were used to perform PCR ribotyping (Fawley et al., 2011) after isolates were sub-cultured in a fastidious anaerobe agar (Bioconnections, Wetherby, UK) and then compared to a panel of the commonest ribotypes circulating in the UK.

In May 2012, the algorithm to diagnose CDI was changed and faecal samples were tested using a combined test for detection of GDH antigen and toxin A/B test in one cartridge (Techlab C. diff Quik Chek Complete). In 2015, a nucleic acid amplification test (NAAT) (Cepheid Xpert® *C. difficile*) was also implemented in this setting as a confirmatory test for those patients who had a negative result for TOX but positive result for GDH. The PCR assay was

used for detection of the toxin gene targeting a species-specific internal fragment of the triose phosphate isomerase (tpi) housekeeping gene, as well as internal core sequences of both tcdA and B tcdB genes to verify their individual toxigenicity (Lemee et al., 2004).

2.2.3 Data collection

All cohort patient data were collected using a Case Report Form (CRF; presented in the appendix 2) and accessing hospital information systems such as Patient Manager (iPM), Integrated Clinical Environment (ICE) and End-to-End E-Prescribing & Medicines Administration (EPMA). Data collected include demographic, clinical and hospitalisation information, laboratory results and medicines. For audit patients, only demographic and hospitalisation data were available.

Demographic information included age at recruitment, gender and index of multiple deprivation 2015 (IMD). Clinical information included the Charlson comorbidity index 2011 (CCI), CDI severity, CDI recurrence and mode of acquisition, and whether the infection was a healthcare-associated infection (HCAI) or community-acquired infection (CAI). Hospitalisation information include length of stay (LoS), disease and pre-test periods, number of hospitalised days within 6 months prior (LoS before) and 6 months post (LoS after) the index hospitalisation, costs of index hospitalisation, costs of hospitalisations within 6 months prior (costs before) and 6 months post (costs after) the index hospitalisation. Laboratory results included the white cell count (WCC), neutrophils, albumin, estimated glomerular filtration rate (eGFR) and

C-reactive protein (CRP). Medicines information included the antibiotics used for CDI treatment and information about proton-pump inhibitors (PPI), a medication for chronic use and potential risk factor for development of CDI.

IMD is a measurement that relates deprivation of hospitalisation, employment, health and disability, education skills and training, barriers to housing and services, living environment and crime by Lower layer Super Output Area (LSOA) (Department For Communities And Local Government, 2011). England is divided into 32,844 LSOA and each LSOA has a minimum of 1,000 residents (average of 1,600) and 400 households (average of 650). IMD was derived using the patients' post codes and Department for Communities and Local Government datasets updated in 2015 (Department For Communities And Local Government, 2015).

CCI is a measurement tool developed to classify and weight comorbid conditions. It predicts ten-year mortality for each patient (Charlson et al., 1987) and takes into account comorbidities such as cardiovascular disease, renal disease, liver disease, cancer and acquired immune deficiency syndrome (AIDS). CCI was derived from CRF notes of comorbidities and history of past diseases and classified according to the recommendations by Quan et al (Quan et al., 2011), which is an updated version of Charlson (Charlson et al., 1987).

HCAI was defined as an infection acquired and developed in healthcare settings as a result of medical interventions or contact with the environment (PHE, 2012). An infection was assumed to be HCAI if the TOX test was performed at least 48 hours after admission to the hospital (Friedman et al,

2002, Gupta and Khanna, 2014). Tests performed before 48h of the hospital admission were considered CAI. Medical history of past hospitalisation and healthcare interventions was not considered and for this reason the definition of cases is not accurate.

All continuous variables were presented as median and interquartile range (IQR) while categorical variables were presented as frequency (F) and percentage (P). T-test or ANOVA were employed to compare normally distributed continuous variables, Mann-Whitney U test or Kruskal Wallis were employed to compare non-normally distributed and chi-square test to compare categorical variables.

2.2.4 Definitions of outcomes

LoS or time to discharge was defined as the total period between the patient's admission until the discharge from hospital. This period was divided in two different periods: the disease period comprising the time between the *C. difficile* test (positive for cases and negative for controls) to discharge from hospital, and the pre-test period which was the time between admission to the *C. difficile* test.

All cause-mortality of patients was assessed at different time-points: short-term mortality (within 4 weeks after TOX), long-term mortality (within 1 year after TOX) and during hospitalisation. Time to death within 1 year was considered the time after the *C. difficile* test until date of death.

The severity of CDI was categorised as either severe or not-severe and assessed only in the cohort of patients who tested GDH+/TOX+ and GDH+/TOX-/PCR+. Severe disease was considered when one of the following clinical signs was present: WCC>$20x10^9$/L, temperature>38.5ºC, eGFR<30ml/min/1.73m^2, severe colitis, hypotension, partial or complete ileus, colectomy and toxic mega colon, according to PHE guideline (PHE, 2013) with inclusion of a more strict cut-off for WCC and eGFR levels in substitution of serum creatinine, as defined previously (Swale, 2014). Levels of WCC higher than $20x10^9$/L have been identified as predictor of complicated CDI in systematic reviews (Chakra et al, 2012, Chakra et al, 2014) and basal serum creatinine was not always available and not always possible to calculate the increase in creatinine levels, for this reason eGFR levels were used instead.

Hospitalisation costs were calculated according to Healthcare Resource Group (HRG) codes and based on the national tariff. After hospital discharge, each hospitalisation received codes by the hospital's clinical coders for diseases and interventions performed during the period. These codes were submitted to Secondary Uses Service that assigns an HRG code based on clinical codes and other patient information. Each HRG code was priced by Department of Health according to the national tariff which is based on average clinical and non-clinical costs of a particular procedure from all NHS hospitals in England. Every year, hospitals should collect costs by type of treatment and submit it to the Department of Health (Department of Health, 2012b). These costs include only direct medical and non-medical costs, such as staff, consumables, overheads, capital charges and diagnostic tests charges. Thus, hospitalisation dates of all patients were submitted to the

information centre and finance department in the RLBUHT to obtain the HRG codes and costs of each patient, however not all patients had these information available The total cost of hospitalisation was based on the 2016/2017 national tariff (NHS England, 2016) and adjusted for LoS when the time point was lower than the total hospitalisation period. In addition, for phase II patients who had been treated with FDX – a high cost antibiotic comparing to standard therapy (£1,350 for 10 days treatment) – the treatment cost was added to the hospitalisation costs.

CDI recurrence, including relapse and reinfection, was considered when a patient had a positive result for TOX within 90 days after the index CDI episode (Swale et al., 2014a). A second positive test within 30 days was excluded following recommendation (Surawicz et al., 2013) as tests could remain positive within this period.

Clinical outcomes were presented in the same format as other variables: continuous variables were presented as median and IQR and categorical variables were presented as F and P. To compare variables, the same testes described in section 2.2.3 were employed.

2.3 Responsibility breakdown

The development of the study "*Clostridium difficile*-associated toxin disease: development of a tool to predict individual susceptibility based on environmental and genetic factors" design was carried out by the Principal Investigator (Professor Sir Munir Pirmohamed), the Study Lead (Dr Fabio Miyajima), the Study Administrator (Ms Anita Hanson), the Microbiology

Consultant (Dr Christopher Parry) and the leading Infectious Disease Consultants (Dr Nicholas Beeching and Dr Mike Beadsworth). The clinical audit was submitted by Dr Mike Beadsworth.

The recruitment of patients and collection of information for CRF were conducted by Pharmacology Research Nurses (Mrs Margaret Little and Ms Rachel Hornby). The electronic version of CRFs was developed by Dr Fabio Miyajima, Dr Andrew Swale, Mr James McKenna and Mr Jon Creswell. Further appropriate data of recruited patients were collected by Mrs Margaret Little, Ms Rachel Hornby, Dr Andrew Swale, Dr Fabio Miyajima and myself. Clinical and hospitalisation information of audit patients were collected by Ms Kathryn Mcgregor, Ms Hannah Richards, Ms Anne Wiltshire and myself.

Blood samples were processed by Dr Andrew Swale and Ms Alejandra Doce Carracedo. Faecal samples were processed by Mr Paul Roberts and Ms Valerie Price at LCL who also performed the microbiological profile. Additional microbiological tests were performed by Ms Zolal Hekmat, Mr Leandro Carneiro and Ms Qing Zhang. Procalcitonin measurement was performed by Dr Suzannah Phillips and Ms Jean Devine at LCL.

Hospitalisation codes were obtained by Mr Paul Currie from The Corporate Information Department and hospitalisation costs were obtained by Colin Duckworth from Finance Department at RLBUHT.

2.4 Results

2.4.1 Overview of main patients' characteristics

Figure 2.1 shows when (phase I or II) and how (cohort or audit) patients were recruited between 2008 and 2016 and Table 2.1 shows the number of patients recruited by diagnostic tests performed and phase of recruitment. In the cohort, 257 CDI cases and 139 diarrhoea control patients were recruited during phase I, while 70 CDI cases, 47 potential carrier patients and 84 diarrhoea control patients were included during phase II. Clinical audit included 416 CDI case patients in the phase I and 171 CDI case patients, 428 potential carrier patients and 3,658 diarrhoea control patients in phase II.

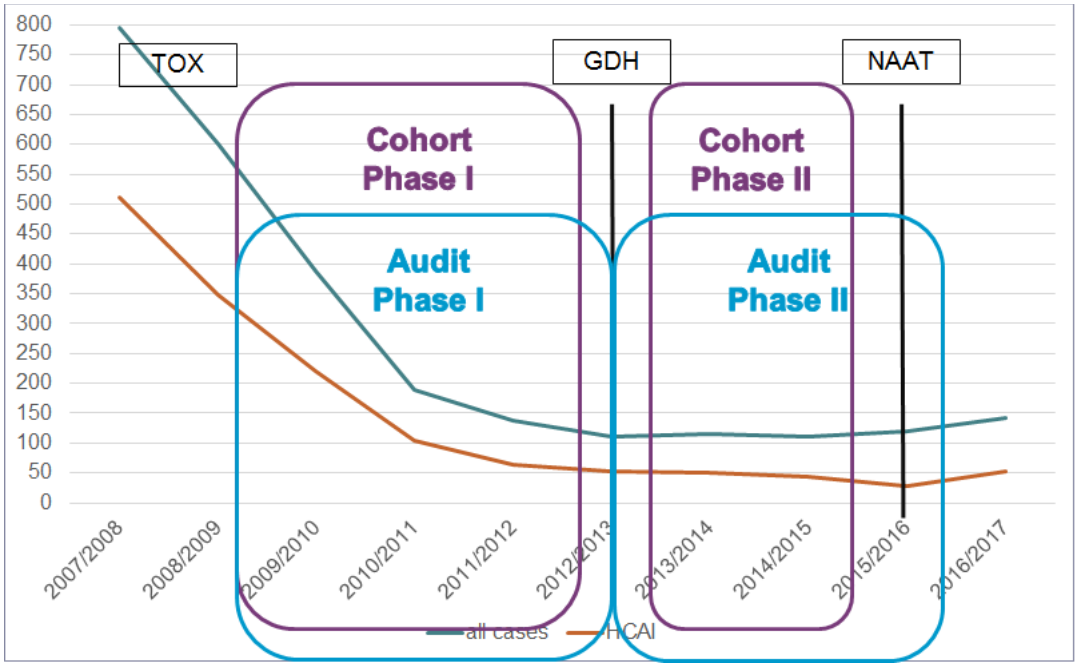

Figure 2.1 Recruitment of patients between 2008 and 2016

Table 2.1 Patients recruited by phase, diagnosis tests and recruitment status

Period	Phase	Diagnosis tests	Patients recruited		
				cohort	audit
2008-2012	I	TOX	TOX-[b]	139	.
			TOX+[c]	257	416
2013-2015[a] **2012-2016**	II	GDH TOX	GDH-/TOX-[d]	164	3,658
			GDH+/TOX-[e]	57	428
			GDH+/TOX+[f]	78	171

TOX: toxin test, GDH: glutamate dehydrogenase
[a] Cohort patients were recruited between 2013 and 2015 and audit patients were recruited between 2012 and 2016 [b] diarrhoea control patients [c] CDI case patients [d] diarrhoea control patients [e] potential carrier patients [f] CDI case patients.

2.4.2 Patient Cohorts

2.4.1.1 Phase I recruitment

Patients' characteristics are shown in Table 2.2. Both groups were on average older than 65 years, a potential risk factor for the disease. CDI patients stayed hospitalised longer [24 days (IQR: 12-46), p=0.001], and the costs of their hospitalisation was higher [£4,563 (IQR: £3,394-8,789), p=0.001] than controls. Mortality rates were also higher during hospitalisation [9% (n=24), p=0.047] and within 1 year [32% (n=81), p=0.002]. 42% (n=107) of CDI patients presented severe disease (as described in section 2.2.4) and 18% (n=47) had recurrence within 90 days. Patients categorised as CDI cases typically presented with leucocytosis (WCC>11x10^9/L), neutrophilia (neutrophils>7.5x10^9/L) and hypoalbuminemia (albumin<35g/L) and both groups presented with elevated CRP levels (CRP>5mg/L). In terms of drug intake, 68% (n=173) of CDI cases were treated with any PPI compared to 58% (n=80) of diarrhoea controls. Additionally, 94% (n=241) of CDI patients were treated with MTZ and/or VAN after a positive test for TOX.

Table 2.2 Patients characteristics of cohort phase I

	TOX-[a] (n=139)		TOX+[b] (n=257)		
	n	Median (IQR) F(P)	n	Median (IQR) F(P)	p
Demographics					
Age (years), median (IQR)	139	67 (57-78)	257	75 (61-81)	0.011
gender (female, %)	139	78 (56)	257	149 (58)	0.721
IMD (score), median (IQR)	135	37.9 (20.4-58.0)	229	34.2 (17.3-56.3)	0.438
Clinical					
CCI (score), median (IQR)	139	1 (0-2)	256	1 (0-2)	0.270
mode of acquisition (HCAI, %)	139	87 (62.6)	257	161 (62.6)	0.991
CDI severity (%)	0		257	107 (41.6)	.
CDI recurrence (%)	0		257	47 (18.3)	.
Clinical outcomes					
LoS (days), median (IQR)	139	14 (7-28)	257	24 (12-46)	0.001
disease (days), median (IQR)	139	7 (4-15)	257	14 (8-27)	0.002
pre-test (days), median (IQR)	139	4 (1-10)	257	7 (1-17)	0.018
Time to death (days), median (IQR)	24	141.5 (44-199.5)	81	79 (31-159)	0.146
Mortality hospitalisation (%)	139	5 (3.6)	257	24 (9.3)	0.047
4 weeks (%)	139	5 (3.6)	257	18 (7.0)	0.167
1 year (%)	139	24 (17.3)	257	81 (31.5)	0.002
Hospitalisation costs (£), median (IQR)	132	3,221 (2,116-5,000)	217	4,563 (3,394-8,789)	0.001
Laboratory results, median (IQR)					
albumin baseline (g/L)	88	34 (29-39)	212	30 (25-35)	<0.001
WCC baseline (10^9/L)	129	8.6 (6.6-12.3)	252	11.6 (8.5-18.0)	0.001
Neutrophils baseline (10^9/L)	126	6.0 (4.2-9.7)	247	9.0 (6.0-15.0)	<0.001
eGFR baseline (mL/min/1.73m^2)	129	71.0 (33.0-95.0)	251	74.0 (46.0-106.0)	0.104
CRP baseline (mg/L)	129	25 (8-85)	240	70.5 (30.5-137.5)	0.002
Medicines (%)					
PPI	139	80 (57.6)	255	173 (67.8)	0.042
CDI treatment[c]	139	14 (10.1)	257	241 (93.8)	<0.001
Fidaxomicin	139	0	257	0	.
Vancomycin	139	6 (4.3)	257	228 (88.7)	<0.001
Metronidazole	139	10 (7.2)	257	111 (43.2)	0.062

TOX: toxin test, IMD: Index of multiple deprivation, CCI: Charlson Comorbidity Index, HCAI: healthcare-associated infection, CDI: *Clostridium difficile* Infection, LoS: length of stay, WCC: white cell count, eGFR: estimated glomerular filtration rate, CRP: C-reactive protein, PCT: procalcitonin, PPI: Proton-pump inhibitor.

[a] diarrhoea control patients, [b] CDI case patients, [c] treatment with any antibiotic (standard treatment or fidaxomicin)

2.4.1.2 Phase II recruitment

Table 2.3 shows characteristics of patients recruited during phase II. The median age of CDI cases in phase II was higher than 65 years. A higher median score of comorbidities was observed in CDI cases (2), followed by GDH+/TOX- (1) and GDH-/TOX- patients (0, p<0.001). Similar clinical outcomes were found in CDI cases and GDH+/TOX- groups when considering the average time to discharge and costs of hospitalisation [16 (IQR: 7-32) vs 18 (IQR: 7-37) days and £5,192 (IQR: £3,842-7,379) vs £5,192 (IQR: £3,144-1,6827], however, the LoS of both groups was higher than diarrhoea control patients (Los 7 days, IQR: 2-10, p>0.001) and costs were similar [£2,971, (IQR: 1,894-3,842), p=0.095]. CDI cases were more likely to die during hospitalisation [8% (n=6), p=0.032] and within 1 year after diagnosis [41% (n=32), p<0.001] compared to the other groups. Hypoalbuminemia (albumin<35g/L) and neutrophilia (neutrophils>7.5x10^9/L) were present in CDI cases and GDH+/TOX- patients. Serum levels of CRP were elevated (CRP>5mg/L) in all groups but levels were higher in the case group [61.5mg/L (IQR: 36.5-195.5), p=0.008]. On average, 72% (n=56) of the CDI group, 63% (n=36) of the GDH+/TOX- group and 47% (n=76) of the control group were treated with a PPI (p=0.001). 92% (n=72) of the CDI patients were treated with standard treatment or FDX whilst 65% (n=37) of GDH+/TOX- patients received antibiotics commonly used for CDI treatment.

Table 2.3 Patients characteristics of cohort phase II

	GDH-/TOX-[a] (n=164)		GDH+/TOX-[b] (n=57)		GDH+/TOX+[c] (n=78)		
	n	Median (IQR) F(P)	n	Median (IQR) F(P)	n	Median (IQR) F(P)	p
Demographics							
Age (years), median (IQR)	164	65 (55.5-72.0)	57	61 (50-72)	78	66.5 (56-79)	0.101
gender (female, %)	164	85 (51.8)	57	30 (52.6)	78	35 (44.9)	0.550
IMD (score), median (IQR)	146	30.5 (16-54)	50	26.2 (11.4-54.8)	75	29.9 (17.3-49.2)	0.870
Clinical							
CCI (score), median (IQR)	164	0 (0-1)	57	1 (0-2)	78	2 (0-3)	<0.001
mode of acquisition (HCAI, %)	164	106 (64.6)	57	33 (57.9)	78	39 (50.0)	0.092
CDI severity (%)	0		29	7 (24.1)	78	34 (43.6)	0.066
CDI recurrence (%)	0				78	7 (9.0)	.
Clinical outcomes							
LoS (days), median (IQR)	109	7 (2-10)	55	18 (7-37)	77	16 (7-32)	<0.001
disease (days), median (IQR)	85	6 (4-9)	55	13 (5-23)	77	10 (5-20)	<0.001
pre-test (days), median (IQR)	85	1 (1-3)	55	4 (1-12)	77	2 (1-12)	0.005
Time to death (days), median (IQR)	6	129 (24-170)	16	127 (88-192)	32	113.5 (46-201)	0.777
Mortality hospitalisation (%)	164	2 (1.2)	57	2 (3.5)	78	6 (7.7)	0.032
4 weeks (%)	164	2 (1.2)	57	1 (1.8)	78	3 (3.8)	0.391
1 year (%)	164	6 (3.7)	57	16 (28.1)	78	32 (41.0)	<0.001
Hospitalisation costs (£), median (IQR)	84	2,971 (1,894-3,842)	47	5,192 (3,144-16,827)	69	5,192 (3,842-7,379)	0.095
Laboratory results							
albumin baseline (g/L)	82	38 (34-41)	51	34 (28-37)	71	31 (27-36)	<0.001
WCC baseline (10^9/L)	88	10 (7.4-13.2)	56	7.8 (5.4-11.0)	77	10.8 (7.1-13.9)	0.711
Neutrophils baseline (10^9/L)	88	7.3 (4.9-10.4)	55	7.8 (4.6-13.0)	74	7.8 (4.6-13.0)	<0.001
eGFR baseline (mL/min/1.73m^2)	87	81.0 (60.0-90.0)	56	77.0 (50.0-90.0)	77	59.0 (39.0-84.0)	0.001
CRP baseline (mg/L)	84	23.5 (8.5-64.5)	44	49 (19.5-97.0)	64	61.5 (36.5-195.5)	0.008

Table 2.3 (continued) Patients characteristics of cohort phase II

	GDH-/TOX-[a] (n=164)		GDH+/TOX-[b] (n=57)		GDH+/TOX+[c] (n=78)		
	n	Median (IQR) F(P)	n	Median (IQR) F(P)	n	Median (IQR) F(P)	p
Medicines (%)							
PPI	161	76 (47.2)	57	36 (63.2)	78	56 (71.8)	0.001
CDI treatment[d]	164	5 (3.1)	57	37 (64.9)	78	72 (92.3)	<0.001
Fidaxomicin	164	0	57	26 (45.6)	78	51 (65.4)	<0.001
Vancomycin	164	0	57	8 (14.0)	78	24 (30.8)	<0.001
Metronidazole	164	5 (3.1)	57	12 (21.1)	78	22 (28.2)	<0.001

GDH: glutamate dehydrogenase, TOX: toxin test, IMD: Index of multiple deprivation, CCI: Charlson Comorbidity Index, HCAI: healthcare-associated infection, CDI: *Clostridium difficile* Infection, LoS: length of stay, WCC: white cell count, eGFR: estimated glomerular filtration rate, CRP: C-reactive protein, PCT: procalcitonin, PPI: Proton-pump inhibitor.

[a] diarrhoea control patients, [b] potential carrier patients, [c] CDI case patients, [d] treatment with any antibiotic (standard treatment or fidaxomicin)

2.4.2 Audit patients

2.4.2.1 Phase I time-period

CDI case patients included in the clinical audit (Table 2.4) had a median age above 65 years, were hospitalised for an average of 29 days (IQR: 3-56.5), and incurred costs of £4,387 (IQR: £3,507-9,367) as a result of hospitalisation. In terms of mortality, 32% (n=135) of the patients died within 4 weeks after the diagnosis, 62% (n=256) within 1 year and 38% (n=157) during hospitalisation.

Table 2.4 Patients characteristics of audit phase I

TOX+[a] (n=416)		
	n	Median (IQR)/F(P)
Demographics		
Age (years), median (IQR)	416	79 (70-87)
gender (female, %)	416	240 (57.7)
IMD (score), median (IQR)	412	40.67 (25.6-59.4)
Clinical		
mode of acquisition (HCAI, %)	416	297 (71.4)
CDI recurrence (%)	416	56 (13.5)
Clinical outcomes		
LoS (days), median (IQR)	416	29 (13-56)
disease (days), median (IQR)	416	14 (6-31)
pre-test (days), median (IQR)	416	9 (2-22)
Time to death (days), median (IQR)	256	27 (676)
Mortality hospitalisation (%)	416	157 (37.7)
4 weeks (%)	416	135 (32.5)
1 year (%)	416	256 (61.5)
Hospitalisation costs (£), median (IQR)	416	4,387 (3,507-9,367)

TOX: toxin test, IMD: Index of Multiple Deprivation, HCAI: healthcare-associated infection, LoS: length of stay

[a] CDI case patients

2.4.2.2 Phase II time-period

All three groups of audit patients (Table 2.5) were on average older than 65 years of age, but CDI group was older than the others [77 years (IQR: 63-84), p<0.001]. The median LoS of the CDI case group was higher than that of the GDH+/TOX- group [18.5 (IQR: 8-36) vs 17 (IQR: 9-34) days, p<0.001] but the costs of hospitalisation were similar [£5,192 (IQR: £3,408-7,406) vs £4,243 (IQR: £3,190-7,325) vs £3,630 (IQR: £2,241-5,205), p=0.179]. The mortality rates were also higher for CDI cases: 25% (n=43) during hospitalisation, 25% (n=43) within 4 weeks, 58% (n=100) within 1 year after diagnosis, p-value for all outcomes were lower than 0.001 comparing the three groups. Laboratory results and use of medicines were collected for only a random sample of control patients. All groups presented with hypoalbuminemia (albumin<35g/L), an elevated level of serum CRP (CRP>5mg/L) and CDI cases with leucocytosis (WCC>11x10^9/L) and neutrophilia (neutrophils>7.5x10^9/L). The use of any PPI was similar in all groups and use of CDI treatments were higher in CDI cases 87% (n=148) compared to potential carrier group 40% (n=172, p<0.001).

Table 2.5 Patients characteristics of audit phase II

	GDH-/TOX-[a] (n=3,658)		GDH+/TOX-[b] (n=428)		GDH+/TOX+[c] (n=171)		
	n	Median (IQR) F(P)	n	Median (IQR) F(P)	n	Median (IQR) F(P)	p
Demographics							
Age (years), median (IQR)	3,658	68 (53-79)	428	70 (53-81)	171	77 (63-84)	<0.001
gender (female, %)	3,658	1,866 (51.0)	428	237 (55.4)	171	102 (59.6)	0.026
IMD (score), median (IQR)	3,545	38.9 (21.4-58.6)	397	42.2 (21.7-58.6)	149	45.7 (25.9-63.7)	0.134
Clinical							
mode of acquisition (HCAI, %)	3,658	1,733 (47.4)	428	231 (54.0)	171	100 (58.5)	0.001
CDI recurrence (%)	0		0		171	16 (9.4)	.
Clinical outcomes							
LoS (days), median (IQR)	3,646	11 (5-24)	399	17 (9-34)	162	22 (10-44)	<0.001
disease (days), median (IQR)	3,646	6 (3-14)	399	10 (5-23)	162	12 (5-25)	<0.001
pre-test (days), median (IQR)	3,646	2 (1-8)	399	3 (1-12)	162	6 (1-17)	<0.001
Time to death (days), median (IQR)	1,043	55 (14-149)	176	52 (18-123)	100	38 (10-111)	0.358
Mortality hospitalisation (%)	3,658	60 (1.6)	428	60 (14.0)	171	43 (25.2)	<0.001
4 weeks (%)	3,658	403 (11.0)	428	67 (15.6)	171	43 (25.2)	<0.001
1 year (%)	3,658	1,043 (28.5)	428	176 (41.1)	171	100 (58.5)	<0.001
Hospitalisation costs (£), median (IQR)	3,618	3,630 (2,241-5,205)	335	4,243 (3,190-7,325)	133	5,192 (3,842-7,406)	0.179
Laboratory results, mean (IQR)							
albumin baseline (g/L)	150	33.0 (26.0-38.0)	351	32.0 (26.0-38.0)	139	28.0 (24.0-32.0)	<0.001
WCC baseline (10^9/L)	186	9.1 (6.5-13.1)	417	9.6 (6.6-13.2)	170	11.3 (7.9-19.0)	<0.001
Neutrophils baseline (10^9/L)	185	6.8 (4.4-10.0)	410	7.3 (4.6-10.4)	162	9.0 (6.0-16.3)	<0.001
eGFR baseline (mL/min/1.73m^2)	164	69.0 (46.0-90.0)	380	67.0 (36.0-90.0)	152	63.5 (34.0-90.0)	0.102
CRP baseline (mg/L)	103	42.0 (11.0-91.0)	249	57.0 (18.0-127.0)	97	84.0 (36.0-170.0)	<0.001

Table 2.5 (continued) Patients characteristics of audit phase II

	GDH-/TOX-[a] (n=3,658)		GDH+/TOX-[b] (n=428)		GDH+/TOX+[c] (n=171)		
	n	Median (IQR) F(P)	n	Median (IQR) F(P)	n	Median (IQR) F(P)	p
Medicines (%)							
PPI	189	104 (55.0)	428	226 (52.8)	171	87 (50.9)	0.731
CDI treatment[d]	189	0	428	172 (40.2)	171	148 (86.6)	<0.001
Fidaxomicin	189	0	428	120 (28.0)	171	101 (59.1)	<0.001
Vancomycin	189	0	428	40 (9.4)	171	43 (25.2)	<0.001
Metronidazole	189	0	428	29 (6.8)	171	32 (18.7)	<0.001

TOX: toxin test, IMD: Index of Multiple Deprivation, HCAI: healthcare-associated infection, LoS: length of stay, WCC: white cell count, eGFR: estimated glomerular filtration rate, CRP: C-reactive protein, PPI: Proton-pump inhibitor, CDI: *Clostridium difficile* infection.
[a] diarrhoea control patients, [b] potential carrier patients, [c] CDI case patients, [d] treatment with any antibiotic (standard treatment or fidaxomicin)

2.4.3 Comparison of CDI patients between cohorts and audits

2.4.3.1 Cohort phase I vs Cohort phase II

Comparison between patients recruited during phase I and II is shown in Table 2.6. Phase I were older [75 years (IQR: 61-81) vs 66 years (IQR: 56-79), p=0.037), with a lower CCI score [1 (IQR: 0-2) vs 2 (IQR: 0-3), p=0.037] and with a higher proportion of female [58% (n=149) vs 45% (n=35), p=0.042] and HCAI [63% (n=161) vs 50% (n=39), p=0.046] than patients recruited during phase II. Although mortality rates were similar, time to death within 1 year after diagnosis was lower for the phase I cohort [81 days (IQR: 31-159) vs 114 days (IQR: 46-201), p=0.038]. Serum levels of WCC, neutrophils and eGFR were also higher in the phase I cohort and the pattern of antibiotic use changed in 2011 when FDX was approved to be used as a CDI treatment.

Table 2.6 Comparison between CDI case patients of cohort phase I and cohort phase II

	Cohort I		Cohort II		
	n	Median (IQR) F(P)	n	Median (IQR) F(P)	p
Demographics					
Age (years), median (IQR)	257	75 (61-81)	78	66.5 (56-79)	0.037
gender (female, %)	257	149 (58)	78	35 (44.9)	0.042
IMD (score), median (IQR)	229	34.2 (17.3-56.3)	75	29.9 (17.3-49.2)	0.093
Clinical					
CCI (score), median (IQR)	256	1 (0-2)	78	2 (0-3)	0.037
mode of acquisition (HCAI, %)	257	161 (62.6)	78	39 (50.0)	0.046
CDI severity (%)	257	107 (41.6)	78	34 (43.6)	0.760
CDI recurrence (%)	257	47 (18.3)	78	7 (9.0)	0.050
Clinical outcomes					
LoS (days), median (IQR)	257	24 (12-46)	77	16 (7-32)	0.210
disease (days), median (IQR)	257	14 (8-27)	77	10 (5-20)	0.102
pre-test (days), median (IQR)	257	7 (1-17)	77	2 (1-12)	0.930
Time to death (days), median (IQR)	81	79 (31-159)	32	114 (46-201)	0.038
Mortality hospitalisation (%)	257	24 (9.3)	78	6 (7.7)	0.729
4 weeks (%)	257	18 (7.0)	78	3 (3.8)	0.314
1 year (%)	257	81 (31.5)	78	32 (41.0)	0.120
Hospitalisation costs (£), median (IQR)	217	4,563 (3,394-8,789)	69	5,192 (3,842-7,379)	0.487
Laboratory results, median (IQR)					
albumin baseline (g/L)	212	30 (25-35)	71	31 (27-36)	0.160
WCC baseline (10^9/L)	252	11.6 (8.5-18.0)	77	10.8 (7.1-13.9)	0.026
Neutrophils baseline (10^9/L)	247	9.0 (6.0-15.0)	74	7.8 (4.6-13.0)	0.023
eGFR baseline (mL/min/1.73m^2)	251	74.0 (46.0-106.0)	77	59.0 (39.0-84.0)	<0.001
CRP baseline (mg/L)	240	70.5 (30.5-137.5)	64	61.5 (36.5-195.5)	0.286
Medicines (%)					
PPI	255	173 (67.8)	78	56 (71.8)	0.552
CDI treatment[c]	257	241 (93.8)	78	72 (92.3)	0.168
Fidaxomicin	257	0	78	51 (65.4)	<0.001
Vancomycin	257	228 (88.7)	78	24 (30.8)	0.050
Metronidazole	257	111 (43.2)	78	22 (28.2)	<0.001

IMD: Index of Multiple Deprivation, CCI: Charlson comorbidity index, HCAI: healthcare-associated infection, CDI: *Clostridium difficile* infection, LoS: length of stay, WCC: white cell count, eGFR: estimated glomerular filtration rate, CRP: C-reactive protein, PPI: Proton-pump inhibitor.

2.4.3.2 Audit phase I vs audit phase II

Comparing audit patients (Table 2.7), as cohort groups, phase I audit had median age (79 years, IQR: 70-87) higher than phase II audit [77 years, (IQR: 63-84), p<0.001) and higher proportion of HCAI [71% (n=297) vs 58% (n=100), p<0.001]. Mean Los [29 days (IQR: 13-56) vs 22 days (IQR: 10-44), p=0.001] and long-term mortality [62% (n=256) vs 59% (n=100), p=0.040] were higher during phase I, but costs of hospitalisation [£4,387 (IQR: 3,507-9,367) vs £5,192 (IQR: 3,842-7,406), p=0.047] were lower compared to phase II.

Table 2.7 Comparison between CDI case patients of audit phase I and audit phase II

	Audit I		**Audit II**		
	n	Median (IQR) F(P)	n	Median (IQR) F(P)	p
Demographics					
Age (years), median (IQR)	416	79 (70-87)	171	77 (63-84)	<0.001
gender (female, %)	416	240 (57.7)	171	102 (59.6)	0.728
IMD (score), median (IQR)	412	40.7 (25.6-59.4)	149	45.7 (25.9-63.7)	0.838
Clinical					
CCI (score), median (IQR)	.	.	.	.	.
mode of acquisition (HCAI, %)	416	297 (71.4)	171	100 (58.5)	<0.001
CDI severity (%)	.	.	.	.	.
CDI recurrence (%)	416	56 (13.5)	171	16 (9.4)	0.168
Clinical outcomes					
LoS (days), median (IQR)	416	29 (13-56)	162	22 (10-44)	0.001
disease (days), median (IQR)	416	14 (6-31)	162	12 (5-25)	0.122
pre-test (days), median (IQR)	416	9 (2-22)	162	6 (1-17)	<0.001
Time to death (days), median (IQR)	256	27 (6-76)	100	38 (10-111)	0.001
Mortality hospitalisation (%)	416	157 (37.7)	171	43 (25.2)	0.353
4 weeks (%)	416	135 (32.5)	171	43 (25.2)	0.333
1 year (%)	416	256 (61.5)	171	100 (58.5)	0.040
Hospitalisation costs (£), median (IQR)	416	4,387 (3,507-9,367)	133	5,192 (3,842-7,406)	0.047

IMD: Index of Multiple Deprivation, CCI: Charlson comorbidity index, HCAI: healthcare-associated infection, CDI: *Clostridium difficile* infection, LoS: length of stay.

2.4.3.3 Cohort phase I vs audit phase I

Comparing audit patients and cohort patients phase I (Table 2.8), non-recruited patients were older [79 years (IQR: 70-87) vs 75 years (IQR: 61-81), p<0.001] and with a higher proportion of HCAI [71% (n=297) vs 63% (n=161), p=0.018] and higher IMD score [40.7 (IQR: 25.6-59.4 vs 34.2 (IQR: 17.3-56.3), p=0.017]. Short-term, long-term and during hospitalisation mortality rates were four-times [32% (n=135) vs 7% (n=18), p<0.001), twice [62% (n=256) vs 32% (n=81), p<0.001] and four-times [38% (n=157) vs 9% (n=23), p<0.001] as high as cohort patients.

Table 2.8 Comparison between CDI case patients of cohort phase I and audit phase I

	Cohort I		**Audit I**		
	n	Median (IQR) F(P)	n	Median (IQR) F(P)	p
Demographics					
Age (years), median (IQR)	257	75 (61-81)	416	79 (70-87)	<0.001
gender (female, %)	257	149 (58)	416	240 (57.7)	0.942
IMD (score), median (IQR)	229	34.2 (17.3-56.3)	412	40.7 (25.6-59.4)	0.017
Clinical					
CCI (score), median (IQR)	256	1 (0-2)	.	.	.
mode of acquisition (HCAI, %)	257	161 (62.6)	416	297 (71.4)	0.018
CDI severity (%)	257	107 (41.6)	.	.	.
CDI recurrence (%)	257	47 (18.3)	416	56 (13.5)	0.091
Clinical outcomes					
LoS (days), median (IQR)	257	24 (12-46)	416	29 (13-56)	0.911
disease (days), median (IQR)	257	14 (8-27)	416	14 (6-31)	0.223
pre-test (days), median (IQR)	257	7 (1-17)	416	9 (2-22)	0.074
Time to death (days), median (IQR)	81	79 (31-159)	256	27 (6-76)	0.012
Mortality hospitalisation (%)	257	23 (9.0)	416	157 (37.7)	<0.001
4 weeks (%)	257	18 (7.0)	416	135 (32.5)	<0.001
1 year (%)	257	81 (31.5)	416	256 (61.5)	<0.001
Hospitalisation costs (£), median (IQR)	217	4,563 (3,394-8,789)	416	4,387 (3,507-9,367)	0.981

IMD: Index of Multiple Deprivation, CCI: Charlson comorbidity index, HCAI: healthcare-associated infection, CDI: *Clostridium difficile* infection, LoS: length of stay.

2.4.3.4 Cohort phase II vs audit phase II

Phase II audit patients compared to cohort patients (Table 2.9) also had higher median age [77 years (IQR: 63-84) vs 66 years (IQR: 56-79), p=0.007] and higher median IMD score [45.7 (IQR: 25.9-63.7) vs 29.9 (IQR: 17.3-49.2), p=0.001] compared to cohort patients. Short [25% (n=43) vs 4% (n=3), p<0.001] and long-term mortality [58% (n=100) vs 41% (n=32), p=0.010] and mortality during hospitalisation [25% (n=43) vs 8% (n=6), p=0.001] were also higher in audit patients. Levels of WCC and neutrophils were more elevated in audit patients and levels of albumin were lower when compared to recruited patients. CDI treatment was similar in both groups but PPI use was higher in cohort patients.

2.5 Discussion

This study was conducted at RLBUHT over two periods, epidemic (2008-2012) and endemic (2013-2015) phases. In total, 335 CDI case patients were recruited and the main difference found between them was in the mode of acquisition of bacteria, laboratory results and CDI treatment. Although clinical outcomes did not differ statistically, phase I patients had on average longer hospitalisation, higher mortality rates and incurred lower hospitalisation costs.

A study conducted in 6 UK hospitals in 2013 and 2014 (Wilcox et al., 2017) showed similar results considering CDI severity (41% for patients with first

Table 2.9 Comparison between CDI case patients of cohort phase II and audit phase II

	Cohort II		**Audit II**		
	n	Median (IQR) F(P)	n	Median (IQR) F(P)	p
Demographics					
Age (years), median (IQR)	78	66.5 (56-79)	171	77 (63-84)	0.007
gender (female, %)	78	35 (44.9)	171	102 (59.6)	0.030
IMD (score), median (IQR)	75	29.9 (17.3-49.2)	149	45.7 (25.9-63.7)	0.001
Clinical					
CCI (score), median (IQR)	78	2 (0-3)	.	.	.
mode of acquisition (HCAI, %)	78	39 (50.0)	171	100 (58.5)	0.211
CDI severity (%)	78	34 (43.6)	.	.	.
CDI recurrence (%)	78	7 (9.0)	171	16 (9.4)	0.923
Clinical outcomes					
LoS (days), median (IQR)	77	16 (7-32)	162	22 (10-44)	0.813
disease (days), median (IQR)	77	10 (5-20)	162	12 (5-25)	0.774
pre-test (days), median (IQR)	77	2 (1-12)	162	6 (1-17)	0.910
Time to death (days), median (IQR)	32	113.5 (46-201)	100	38 (10-111)	0.513
Mortality hospitalisation (%)	78	6 (7.7)	171	43 (25.2)	0.001
4 weeks (%)	78	3 (3.8)	171	43 (25.2)	<0.001
1 year (%)	78	32 (41.0)	171	100 (58.5)	0.010
Hospitalisation costs (£), median (IQR)	69	5,192 (3,842-7,379)	133	5,192 (3,842-7,406)	0.128
Laboratory results, median (IQR)					
albumin baseline (g/L)	71	31 (27-36)	139	28.0 (24.0-32.0)	0.004
WCC baseline (10^9/L)	77	10.8 (7.1-13.9)	170	11.3 (7.9-19.0)	0.009
Neutrophils baseline (10^9/L)	74	7.8 (4.6-13.0)	162	9.0 (6.0-16.3)	0.011
eGFR baseline (mL/min/1.73m^2)	77	59.0 (39.0-84.0)	152	63.5 (34.0-90.0)	0.074
CRP baseline (mg/L)	64	61.5 (36.5-195.5)	97	84.0 (36.0-170.0)	0.979
Medicines (%)					
PPI	78	56 (71.8)	171	87 (50.9)	0.002
CDI treatment[c]	78	72 (92.3)	171	148 (86.6)	0.189
Fidaxomicin	78	51 (65.4)	171	101 (59.1)	0.343
Vancomycin	78	24 (30.8)	171	43 (25.2)	0.353
Metronidazole	78	22 (28.2)	171	87 (50.9)	0.092

IMD: Index of Multiple Deprivation, CCI: Charlson comorbidity index, HCAI: healthcare-associated infection, CDI: *Clostridium difficile* infection, LoS: length of stay, WCC: white cell count, eGFR: estimated glomerular filtration rate, CRP: C-reactive protein, PPI: Proton-pump inhibitor.

episode and 52% for patients with recurrent case) and median LoS (16-21 days). However, overall costs (£6,294-7,539) and proportion of HCAI (81%) were higher than our cohort and audit groups. Median CCI score (1) and LoS (15 days) of French patients infected by *C. difficile* between 2007 and 2014 were also similar compared to our patients (Barbut et al., 2017a). Between 2014 and 2015, a study found similar CCI score (2) and mortality rates within 30 days (18%) when considering both cohorts and audits, however, the proportion of HCAI was also higher (85%) and similar to the study in the UK (Barbut et al., 2017b). Short-term mortality was also reported as 11% in a Japanese cohort (Takahashi et al., 2014).

Biomarker levels in all groups indicated a possible inflammatory process and bacterial infection with hypoalbuminemia (albumin>35g/L), neutrophilia (neutrophils>7.5x10^9/L) and elevated levels of CRP. Between 2006 and 2010, a cohort recruited in a hospital in the UK found similar results with median levels of 11.2x10^9/L for WCC, 8.9x10^9/L for neutrophils, 34g/L for albumin and 84 mg/L for CRP (Eyre et al., 2012). Another study conducted between 2014 and 2015 found lower levels of all these biomarkers: WCC (7.6x10^9/L), neutrophils (7.0x10^9/L), albumin (32g/L) and CRP (45mg/L) (Barbut et al., 2017b).

After submission of the audit, 587 CDI cases were included in this study. Comparing groups from phase I and II, phase I patients had longer hospitalisation, higher long-term mortality rate, lower hospitalisation costs and higher proportion of HCAI. Information about laboratory results and medicines were not collected for phase I audit patients.

The comparison between patients from cohort and audit showed that median age, short and long-term mortality rates and proportion of HCAI were higher in the audit and phase I group patients. The audit patients had a higher IMD score in both phases meaning that those patients were living in more deprived areas.

The difference between phases can be explained by the virulent strain RT027 that caused numerous outbreaks between 2006 and 2008 and it was associated with severe illness and increased death and recurrence rates (PHE, 2017). During this period, there was an increase of over 4-fold in the number of associated deaths (CDC, 2013). The difference between cohort and audit patients may be explained by inclusion and exclusion criteria of recruitment as the patient or a personal nominated consultee needed to be able to give informed consent to include the patient in the study. For instance, during phase I, 877 CDI cases were not recruited because: patients were unable to consent (414), were not admitted (229), were previously recruited (75), were discharged or died (63), decline to participate (49) or other reason (47) (Swale, 2014).

The proportion of community-acquired infections increased in both phase II cohort and audit patients compared to phase I. Although the proportion of HCAI in our setting was lower than those found in published papers, this finding correlates with national figures that show that since 2010 the majority of CDI patients were not infected in healthcare settings and in 2015/2016, CAI reached 64% of the total number of cases (PHE, 2017).

FDX was approved to treat CDI patients in 2011 and currently it is the first-line treatment for all patients in this setting. Thus, the use of FDX was only possible to compare during phase II and the pattern of use was similar between cohort and audit patients. Standard treatment with either MTZ or VAN was also similar between these two groups. PPI, a potential risk factor for CDI was taken by at least 50% of all groups. Audit patients presented a lower rate of intake, but this information was collected electronically and only medicines administered during hospitalisations were recorded. For this reason, this rate could be underestimated.

Although there are significant differences between cohort and audit patients, these differences were found in both phases which means that our recruitment excluded more debilitated patients and it may be a selection bias when only considering cohort patients, but some factors are impossible to control and avoid when consent is needed. A large number of patients who were screened to be eligible for recruitment declined to participate, and it is of course, important not to pressure or coerce patients in getting involved in research. Nevertheless, our recruited patients, with the associated biobank, represent a valuable resource by which to study inter-individual variability in both the infecting organism and host susceptibility.

Chapter 3

An evaluation of the toxigenicity of *Clostridium difficile* isolates and clinical outcomes from GDH-positive specimens in a large hospital setting

3.1 Introduction

Samples with a positive GDH test but a negative confirmatory TOX test are found routinely in clinical care, flagging up a new segment of patients characterised by either: *i) C. difficile* colonisation with a non-toxigenic variant; or *ii)* carriage of a toxigenic strain type producing no detectable levels of toxins (Shetty et al., 2011), which in some instances reflects an incipient disease state. Those individuals, so-called potential *C. difficile* 'excretors', may be an important reservoir of the organism in both healthcare settings and the community (Jones et al., 2013).

Such samples ideally require a third screening test to rule out colonization by non-toxigenic strains, thus resolving potential discrepancies and optimizing resources. The test recommended by the Department of Health, to be used in combination with TOX, that could be used as a third and confirmatory diagnostic test is the nucleic acid amplification test (NAAT) for real-time detection of toxin genes (Department of Health, 2012).

The policy in the RLBUHT NHS Trust was to treat all symptomatic patients with FDX, a high cost antibiotic. The identification of the potential *C. difficile* 'excretors' is relevant from a health economic perspective as patients carrying non-toxigenic isolates would not require expensive treatments and isolation measures. Thus, this study has been conducted to (i) investigate clinical outcomes and potential contribution of these individuals (GDH+/TOX-) as a reservoir for nosocomial transmission of CDI in a consecutive group of diarrhoea patients from a large hospital setting in Liverpool and (ii) assess the potential cost savings of implementing a third and confirmatory test.

3.2 Patients and methodology

3.2.1 Patients

Phase II patients with a GDH test result and all audit patients with clinical data were included in this study. Patient recruitment and data collection are described in section 2.2.3.

3.2.2 Laboratory Testing

During the period between May 2012 and January 2016 following the introduction of a two-step diagnostic algorithm, all faecal samples submitted to the medical microbiology laboratory for CDI testing were screened using a combined GDH and TOX test (Techlab C. diff Quik Chek Complete). GDH+ samples were cultured regardless of their TOX results and a multiplex PCR assay was performed to verify toxigenicity and identify ribotypes. NAAT test (Cepheid Xpert® *C. difficile*) was implemented as the third diagnostic test in this setting from 2015 only.

3.3 Statistical Analysis

3.3.1 Patient characteristics

Descriptive analyses were undertaken to assess the differences in demographics, hospitalisation and microbiological characteristics, laboratory results and use of medicines in CDI cases, GDH+/TOX- patients (toxigenic

and non-toxigenic strains) and diarrhoea control patients. The characteristics are described by median and interquartile ranges (IQR) for continuous variables and frequency (F) and proportion (P) for categorical variables. ANOVA was employed to compare normally distributed continuous variables, Kruskal Wallis was employed to compare non-normally distributed continuous variables and the chi-square test was used to compare categorical variables.

3.3.2 Multivariable analysis

A univariate analysis was conducted to identify potential covariates to be included in the multivariable model. Outliers of hospitalisation costs were excluded when patients had an unrelated diagnostic test that resulted in high or low costs. All models were built using forward and backward stepwise regression with use of $p \leq 0.05$ for inclusion and $p > 0.05$ for exclusion of the relevant covariates.

Logistic regression was chosen to analyse mortality rates (within 4 weeks and 1 year) and severity of CDI. Multivariable Cox proportional hazards was performed to assess the time to discharge and time to mortality within 1 year after the TOX test. For time to discharge no censoring was applied, but patients who did not die within 365 days of TOX were censored for this analysis. A generalised linear model (GLM) with gamma distribution and log link was used to assess hospitalisation costs. Two analyses were performed including all patients and only patients infected by toxigenic strains for all clinical outcomes. All statistics were performed using STATA version 14.0

(StataCorp LP, College Station, Texas). Statistical significance was set at p <0.05.

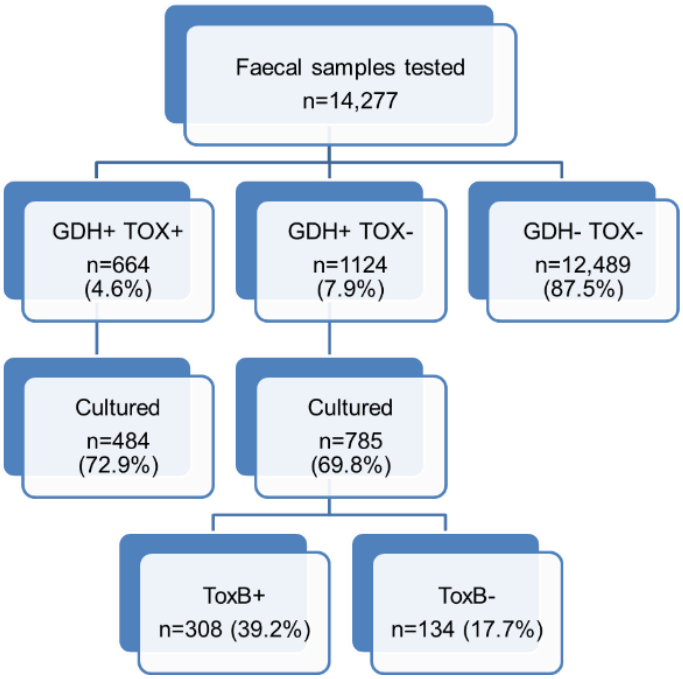

Figure 3.1 Total faecal samples tested between May 2012 and January 2016

3.4 Laboratory profile at Liverpool Clinical Laboratory

During the study period, a total of 14,277 faecal samples from both hospital and community patients were referred for CDI testing (Figure 3.1) at LCL. Of these, 664 (5%) tested GDH+/TOX+ and were confirmed to be CDI cases, 1,124 (8%) had a GDH+/TOX- result and 12,489 (88%) tested negative for both GDH-/TOX-. Hospital patients (Figure 3.2) represented 64% (n=9,166) of the total number of samples, of which 419 (5%) were cases, 710 (8%) had discordant results and 8,037 (88%) were diarrhoea controls. A total of 785 GDH+/TOX- and 484 GDH+/TOX+ samples were cultured in the same period

considering all samples, and 596 and 368 samples, respectively, considering the hospital patients only.

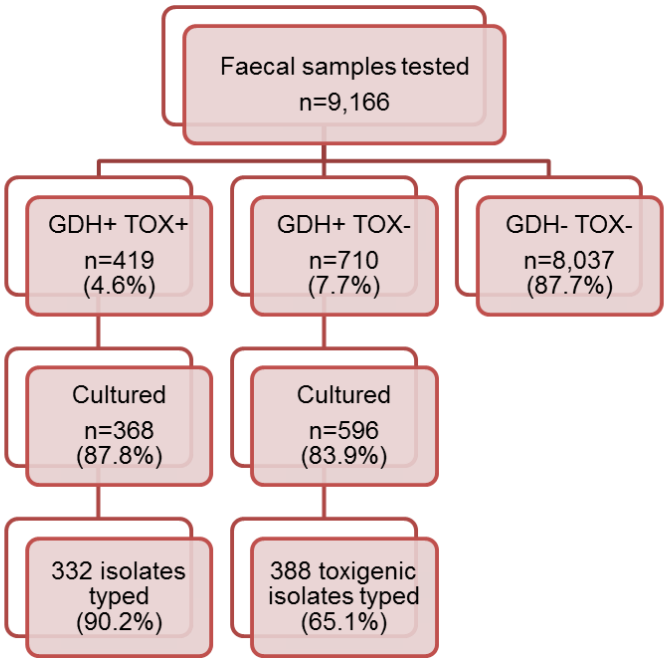

Figure 3.2 Hospital faecal samples tested between May 2012 and January 2016

3.5 Results

3.5.1 Patients characteristics

1,015 patients were selected for this study of which 27% (280) were control patients, 48% (485) were GDH+/TOX- patients and 25% (250) were case patients (Figure 3.3). Of those 485 GDH+/TOX- samples:

- 149 (31%) had a non-toxigenic isolate (GDH+/TOX-/PCR-),
- 237 (49%) contained an isolate possessing the toxin gene (GDH+/TOX-/PCR+); and
- from 99 (20%) patients, it was not possible to get this information and they were excluded from the study.

Thus, considering only patients with PCR results, non-toxigenic samples represented 39% (149/386) and toxigenic samples 61% (237/386) of total GDH+/TOX- samples.

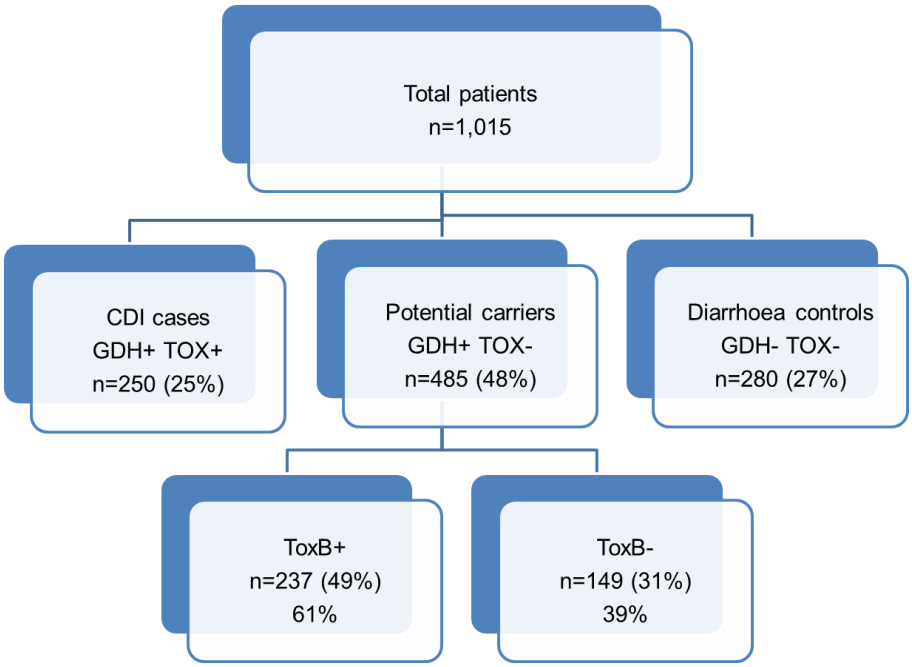

Figure 3.3 Patients recruited in this study by group

Table 3.1 shows patients' characteristics while Table 3.2 shows the differences between all groups and each group compared to CDI cases. The median age of diarrhoea control patients was 65 years (IQR: 51-76) and 48% (n=134) of them were female. Seventy years (IQR: 53-80) and 69 years (IQR: 53-80) were the median ages of the GDH+/TOX-/PCR- and GDH+/TOX-/PCR+ groups. The proportion of females were 56% (n=83) and 57% (n=136), respectively. Case patients were older with a median age of 75 years (IQR: 59-82) and 55% (n=137) were female patients. The four groups were similar in the IMD 2015 score , time to death within 1 year, prior use of proton-pump

inhibitors (PPI), zopiclone and immunosuppressive medicines, the last three being potential risk factors for the development of CDI.

Laboratory results showed that the CDI case group presented with neutrophilia (neutrophils>7.5x10^9/L) and higher white cell counts (WCC) compared to other groups. Hypoalbuminaemia (albumin<35g/L) was seen in all GDH+ groups. CRP levels were above the reference range in all groups (CRP>5mg/L). Thus, case patients presented with laboratory results that were consistent with bacterial infection and an inflammatory process.

GDH+/TOX-/PCR- patients presented with laboratory results that were more similar to CDI cases than those patients whose PCR test was positive. FDX, MTZ and VAN were used to treat 16% (n=24), 5% (n=8) and 8% (n=12) of PCR- patients; 44% (n=104), 8% (n=18) and 8% (n=20) of PCR+ patients; and 61% (n=152), 22% (n=54) and 27% (n=67) of case patients, respectively. Prescription of nutritional supplements with antibiotic therapy is recommended in some NHS healthcare settings and it was used by 26% (n=71) of control, 51% (n=76) of PCR-, 62% (n=149) of PCR+ and 82% (n=205) of case patients.

Table 3.1 Patients characteristics by group

	GDH-/TOX- (n=277)[a]		GDH+/TOX-/ PCR- (n=149)[b]		GDH+/TOX-/ PCR+ (n=239)[c]		GDH+/TOX+ (n=249)[d]	
	n	Median (IQR)/ F(P)	n	Median(IQR)/ F(P)	n	Median(IQR)/ F(P)	n	Median (IQR)/ F(P)
Demographics								
age (years)	277	65 (51-76)	149	70 (53-80)	239	69 (53-80)	249	75 (59-82)
gender (female)	277	134 (48.4)	149	83 (55.7)	239	136 (56.9)	249	137 (55.0)
IMD (score)	257	40.7 (20.8-57.6)	142	43.3 (21.6-61.6)	217	38.8 (21.4-58.6)	224	36.6 (21.4-59.7)
Clinical outcomes								
LoS (days)	261	12 (6-27)	141	20 (10-38)	225	16 (7-34)	239	19 (9-41)
disease (days)	260	8 (4-17)	141	13 (6-28)	225	11 (5-21)	239	12 (5-24)
pre-test (days)	260	2 (1-8)	141	3 (1-15)	225	2 (1-10)	239	4 (1-16)
time to death (days)	57	37 (16-127)	59	58 (18-139)	97	53 (18-139)	132	47.5 (15.5-125.5)
mortality hospitalisation (%)	277	19 (6.9)	149	22 (14.8)	239	28 (11.7)	249	49 (19.7)
mortality 4 weeks (%)	277	26 (9.4)	149	24 (16.1)	239	33 (13.8)	249	46 (18.5)
mortality 1 year (%)	277	57 (20.6)	149	59 (39.6)	239	97 (40.6)	249	132 (53.0)
hospitalisation costs (£)	217	£3,221 (2,442-4,558)	109	£3,842 (3,109-7,599)	179	£4,571 (3,190-6,237)	198	£5,192 (3,842-7,135)
mode of acquisition (HCAI, %)	260	114 (43.8)	141	77 (54.6)	225	104 (46.2)	239	125 (52.3)
CDI severity (%)	0		0		31	7 (22.6)	78	34 (43.6)
CDI recurrence (%)	0		0		0		249	23 (9.2)
Laboratory results, median (IQR)								
albumin baseline (g/L)	231	35 (30-39)	113	32 (26-37)	200	32.5 (27.5-38.0)	210	30 (26-34)
WCC baseline (109/L)	273	9.4 (6.8-13.2)	146	9.5 (6.7-13.9)	233	9.3 (6.6-12.8)	247	11 (7.7-18.2)
Neutrophils baseline (109/L)	272	7.0 (4.5-10.3)	144	7.2 (4.6-11.4)	229	6.9 (4.6-10.0)	236	8.3 (5.7-15.1)
eGFR baseline (mL/min/1.73m2)	250	74.5 (48-90)	135	72 (42-90)	213	64 (34-90)	229	59 (31-89)
CRP baseline (mg/L)	185	33 (10-79)	80	54 (16-130)	149	54 (16-105)	161	77 (36-172)

Table 3.1 (continued) Patients characteristics by group

	GDH-/TOX- (n=277)[a]		GDH+/TOX-/ PCR- (n=149)[b]		GDH+/TOX-/ PCR+ (n=239)[c]		GDH+/TOX+ (n=249)[d]	
	n	Median (IQR)/ F(P)	n	Median(IQR)/ F(P)	n	Median(IQR)/ F(P)	n	Median (IQR)/ F(P)
Medicines (%)								
fidaxomicin	277	0	149	24 (16.1)	239	104 (43.5)	249	152 (61.0)
metronidazole	277	5 (1.8)	149	8 (5.4)	239	18 (7.5)	249	54 (21.7)
vancomycin	277	0	149	12 (8.1)	239	20 (8.4)	249	67 (26.9)
other antibiotic	277	27 (9.8)	149	24 (16.1)	239	15 (6.3)	249	27 (10.8)
nutritional complement	277	71 (25.6)	149	76 (51.0)	239	149 (62.3)	249	205 (82.3)
zopiclone	277	29 (10.5)	149	13 (8.7)	239	25 (10.5)	249	20 (8.0)
PPI	277	159 (57.4)	149	79 (53.0)	239	134 (56.1)	249	143 (57.4)
immunosuppressive	277	39 (14.1)	149	22 (14.8)	239	28 (11.7)	249	22 (8.8)

GDH: Glutamate Dehydrogenase test; TOX: toxin test; IMD: index of multiple deprivation; LoS: length of stay; HCAI: healthcare-associated infection; WCC: white cell count; eGFR: estimated glomerular filtration rate; CRP: C-reactive protein; PPI: Proton-pump inhibitor.
[a] diarrhoea control patients [b] non-toxigenic patients [c] toxigenic patients [d] CDI cases

Table 3.2 Comparison between all groups and between each group and CDI cases

	all groups	GDH- TOX-[a]	GDH+ TOX- PCR-[b]	GDH+ TOX- PCR+[c]
Demographics				
age	<0.001	<0.001	0.025	0.006
gender (female)	0.207	0.128	0.894	0.675
IMD	0.876	0.952	0.484	0.850
Hospitalisation				
LoS	0.028	0.020	0.486	0.292
disease	0.196	0.313	0.118	0.862
pre-test	0.007	0.002	0.634	0.042
time to death	0.782	0.579	0.595	0.661
mortality hospitalisation	<0.001	<0.001	0.215	0.016
4 weeks	0.023	0.002	0.548	0.162
1 year	<0.001	<0.001	0.010	0.006
hospitalisation costs	<0.001	<0.001	0.850	0.028
mode of acquisition	0.102	0.059	0.663	0.191
CDI severity	0.041	.	.	0.041
recurrence	.	.	.	.
Laboratory results				
albumin	<0.001	<0.001	0.004	<0.001
WCC	<0.001	<0.001	0.057	0.001
neutrophils	<0.001	<0.001	0.002	<0.001
eGFR	<0.001	<0.001	0.017	0.377
CRP	<0.001	<0.001	0.100	0.002
Medicines				
fidaxomicin	<0.001	<0.001	<0.001	<0.001
metronidazole	<0.001	<0.001	<0.001	<0.001
vancomycin	<0.001	<0.001	<0.001	<0.001
other antibiotic	0.020	0.679	0.128	0.072
optifibre	<0.001	<0.001	<0.001	<0.001
zopiclone	0.733	0.337	0.808	0.354
PPI	0.820	0.995	0.391	0.761
immunosuppressive	0.210	0.061	0.068	0.294

GDH: Glutamate Dehydrogenase test; TOX: toxin test; IMD: index of multiple deprivation; LoS: length of stay; HCAI: healthcare-associated infection; WCC: white cell count; eGFR: estimated glomerular filtration rate; CRP: C-reactive protein; PPI: Proton-pump inhibitor.

[a] diarrhoea control patients [b] non-toxigenic patients [c] toxigenic patients

3.5.2 Microbiological data

Of 304 isolates cultured from GDH+/TOX- faecal samples in the LCL, 204 (67%) were found to carry genes for toxin B, whilst 100 (32.9%) were deemed non-toxigenic. Among the 193 toxigenic isolates obtained from GDH+/TOX- patients, PCR-ribotype RT014 (16%, n=31) was the most frequently identified, followed by RT002 (10%, n=19), RT020 (10%, n=19), RT015 (8%, n=16), RT078 (6%, n=12) and RT005 (6%, n=12) (Table 3.3).

Among the 223 isolates cultured from CDI patients who tested GDH+/TOX+ ribotype profiles were similar but RT078 (14%, n=31) was the most frequent, followed by RT002 (11%, n=25), RT014 (11%, n=24), RT015 (10%, n=23), RT005 (7%, n=16), and RT020 (3.6%, n=8) (Table 3.3). The ribotypes RT001, RT002, RT003, RT005, RT010, RT012, RT014, RT015, RT017, RT018, RT020, RT023, RT026, RT027, RT046, RT056, RT078, RT081, RT087, and RT106 were also identified in these patients, and were grouped as others (Figure 3.4). RT078 was comparatively more commonly found in stools of patients who tested GDH+/TOX+, but it was also be isolated from the stools of GDH+/TOX- patients, indicating that these individuals can serve as a potential reservoir for these strains. Furthermore, RT014 was more frequently associated with patients who tested GDH+/TOX- than those found to be GDH+/TOX+. From a clinical point of view, there was no consistent pattern to the clinical outcomes with the different ribotypes, although statistically it is possible to observe random significance with a small number of individuals (Table 3.4).

Table 3.3 Ribotypes profile by group

Ribotypes	GDH+/TOX-/PCR+[a]	GDH+/TOX+[b]
RT002	19 (9.8)	25 (11.2)
RT005	12 (6.2)	16 (7.2)
RT014	31 (16.1)	24 (10.8)
RT015	16 (8.3)	23 (10.3)
RT020	19 (9.8)	8 (3.6)
RT027	3 (1.6)	11 (4.9)
RT078	12 (6.2)	31 (13.9)
Other	81 (42.0)	85 (38.1)
Total	193 (100)	223 (100)

[a] carrier patients [b] CDI cases

Table 3.4 Comparison of ribotypes and different outcomes

	Time to discharge		**Mortality 4 weeks**		**Mortality 1 year**		**Time to death**		**CDI severity**		**CDI recurrence**		**Hospitalisation costs**	
	HZ	p-value	OR	p-value	OR	p-value	HZ	p-value	OR	p-value	OR	p-value	expβ	p-value
RT002	1.00		1.00		1.00		1.00		1.00		1.00		1.00	
RT005	0.75	0.290	0.54	0.396	0.52	0.181	0.74	0.382	0.67	0.672	1.05	0.962	1.48	0.037
RT014	0.93	0.764	0.88	0.812	0.58	0.179	1.06	0.830	0.29	0.185	1.05	0.957	1.42	0.024
RT015	1.43	0.165	1.35	0.582	0.48	0.102	1.06	0.853	0.67	0.697	0.70	0.709	1.07	0.683
RT020	1.27	0.365	0.36	0.220	0.24	0.008	0.40	0.030	0.67	0.733	1.00		1.24	0.254
RT027	0.53	0.091	2.50	0.179	1.25	0.730	0.86	0.693	1.00		1.63	0.624	1.38	0.251
RT078	0.71	0.192	1.55	0.405	1.43	0.420	1.06	0.835	0.10	0.022	0.51	0.476	1.17	0.346
Other	0.90	0.585	0.76	0.542	0.57	0.103	0.79	0.311	0.31	0.107	0.66	0.567	1.25	0.080

HZ: hazard ratio; OR: odds ratio; expβ: exponential of β.

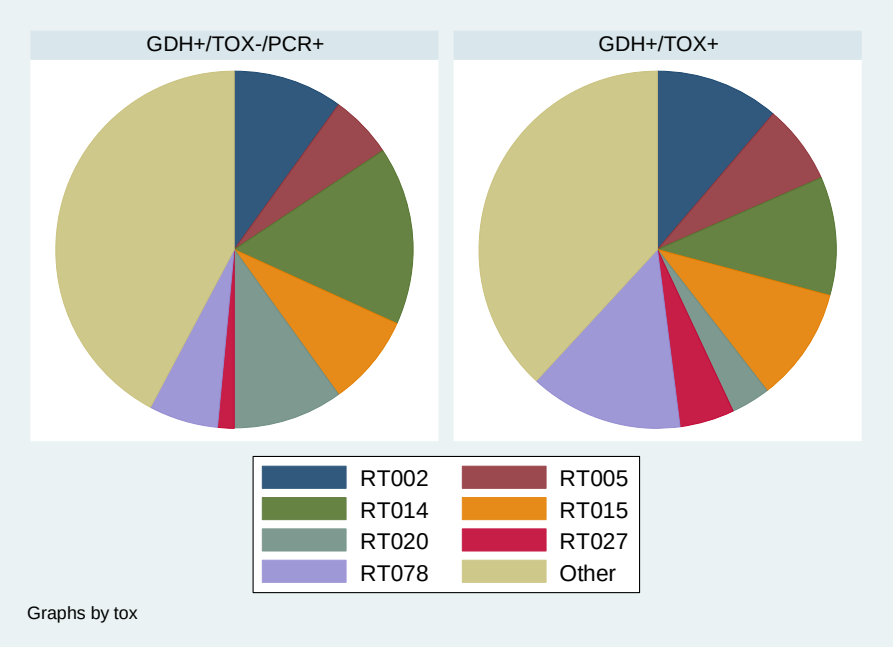

Figure 3.4 Distribution of ribotypes by group

3.5.3 Hospitalisation period/time to discharge

PCR- patients were in hospital [20 days (IQR: 10-38)] for the same amount of time as case patients [19 days (IQR: 9-41)] and PCR+ patients [16 days (IQR: 7-34)], while diarrhoea control patients stayed in hospital on average for 12 days (IQR: 6-27). All groups stayed in hospital for a similar time after the *C. difficile* test [CDI case 12 (IQR: 5-24) vs PCR+ 11 (IQR: 5-21) vs PCR- 13 (IQR: 6-28) vs control 8 days (IQR: 4-17)]. Nevertheless, time to be tested was on average higher in case patients [4 days (IQR: 1-16)] compared to PCR+ [2 days (IQR: 1-10)] and control patients [2 days (IQR: 1-8)] and similar to PCR- patients [3 days (IQR: 1-15)].

A Cox regression analysis for time to discharge was performed using CDI status, age, gender, levels of serum albumin and mode of acquisition as covariates for the initial model (Table 3.5). Variables associated with discharge

rates were GDH+/TOX-/PCR- (HR=0.60, 95% CI: 0.46-0.77), GDH+/TOX-/PCR+ (HR=0.73, 95% CI: 0.59-0.90), CDI cases (HR=0.76, 95% CI: 0.61-0.95), decreased age (HR=0.99, 95% CI: 0.98-0.99), increased levels of serum albumin (HR=1.04, 95% CI: 1.03-1.05), and community-acquired infection (HR=0.34, 95% CI: 0.29-0.41) (Table 3.5).

Table 3.5 Univariate analysis of LoS

LoS	All patients			Toxigenic strains		
	n	Coef (95% CI)	p	n	Coef (95% CI)	p
CDI status	844	2.39 (1.06-3.73)	<0.001	452	2.54 (-2.10-7.19)	0.282
gender	944	-1.01 (-4.00-1.99)	0.509	452	-1.57 (-6.25-3.11)	0.510
age	944	0.16 (0.08-0.23)	<0.001	452	0.12 (-0.01-0.25)	0.069
IMD score	878	0.01 (-0.06-0.08)	0.752	415	0.04 (-0.06-0.15)	0.429
mode of acquisition	933	20.95 (18.25-23.65)	<0.001	452	24.27 (20.20-28.34)	<0.001
CDI severity				102	-1.43 (-11.12-8.27)	0.771
disease	933	1.13 (1.08-1.18)	<0.001	452	1.12 (1.05-1.20)	<0.001
pre-test	933	1.21 (1.13-1.29)	<0.001	452	1.17 (1.06-1.28)	<0.001
mortality 4 weeks	944	-0.60 (-4.84-3.64)	0.780	452	-4.27 (-10.44-1.91)	0.175
mortality 1 year	944	9.09 (6.06-12.11)	<0.001	452	7.81 (3.21-12.41)	0.001
albumin	791	-0.99 (-1.20--0.79)	<0.001	379	-1.12 (-1.46--0.78)	<0.001
WCC	936	-0.02 (-0.19-0.16)	0.831	448	-0.13 (-0.38-0.12)	0.314
neutrophils	919	0.11 (-0.11-0.32)	0.347	436	-0.05 (-0.36-0.25)	0.733
CRP	607	0.01 (-0.01-0.03)	0.248	290	0.01 (-0.02-0.04)	0.472

IMD: index of multiple deprivation; WCC: white cell count; eGFR: estimated glomerular filtration rate; CRP: C-reactive protein.

Considering toxigenic strains, the initial model included the presence of toxin, age, gender, serum levels of albumin and mode of acquisition. The final model found positive association with time to discharge and decreased age (HR=0.99, 95% CI: 0.98-1.00), increased levels of serum albumin (HR=1.06, 95% CI: 1.04-1.08) and community-acquired infection (HR=0.36, 95% CI: 0.28-0.46) (Table 3.6).

Table 3.6 Cox proportional hazards of multivariable analysis for time to discharge

Time to discharge	All patients (n=707)		Toxigenic strains	
	Haz Ratio	p	Haz Ratio	p
GDH-/TOX-[a]	1.00			
GDH+/TOX-/PCR-[b]	0.60 (0.46-0.77)	<0.001		
GDH+/TOX-/PCR+[c]	0.73 (0.59-0.90)	0.004		
GDH+/TOX+[d]	0.76 (0.61-0.95)	0.014		
toxin			1.12 (0.89-1.52)	0.328
age	0.99 (0.98-0.99)	<0.001	0.99 (0.98-1.00)	0.008
mode of acquisition	0.34 (0.29-0.41)	<0.001	0.36 (0.28-0.46)	<0.001
albumin	1.04 (1.03-1.05)	<0.001	1.06 (1.04-1.08)	<0.001

[a] diarrhoea control patients [b] non-toxigenic patients [c] toxigenic patients [d] CDI cases

3.5.4 Mortality rates

3.5.4.1 Short-term mortality (within 4 weeks)

The short-term mortality (within 4 weeks after TOX) was slightly higher for case patients (18%, n=46) compared to PCR+ (14%, n=33), and PCR- (16%, n=24) patients, but was double that seen in control patients (9%, n=26). Causes of death were not accessed in this study, and thus it is not possible to determine if CDI contributed directly or indirectly to the death of those patients.

A logistic regression analysis was performed and covariates included in the initial model were CDI status, gender, age, disease and pre-test periods, levels of WCC, albumin and CRP for mortality within 4 weeks (Table 3.7). Considering only case and colonised patients, toxin, gender, age, disease, levels of WCC, albumin and CRP (Table 3.7), were included in the initial model. The variables gender and toxin were included in the models as they were considered potential confounders while the neutrophil count was excluded from the model due to the high degree of collinearity with WCC.

Table 3.7 Univariate analysis of short-term mortality

Short-term mortality	All patients			Toxigenic strains		
	n	Odds ratio	p	n	Odds ratio	p
CDI	1,015	1.47 (1.15-1.89)	0.003	489	1.41 (0.87-2.29)	0.169
gender	1,015	1.24 (0.86-1.78)	0.245	489	1.27 (0.78-2.08)	0.336
age	1,015	1.04 (1.03-1.05)	<0.001	489	1.03 (1.02-1.05)	<0.001
IMD score	931	1.01 (1.00-1.02)	0.121	441	1.01 (1.00-1.02)	0.103
mode of acquisition	1,015	1.31 (0.92-1.88)	0.137	465	0.96 (0.59-1.57)	0.885
CDI severity				109	3.44 (0.30-	0.320
disease	957	0.97 (0.96-0.99)	<0.001	465	0.96 (0.93-0.98)	<0.001
pre-test	957	1.01 (1.00-1.01)	0.042	465	1.01 (1.00-1.02)	0.111
albumin	847	0.87 (0.84-0.90)	<0.001	411	0.89 (0.85-0.93)	<0.001
WCC	997	1.05 (1.03-1.07)	<0.001	481	1.04 (1.01-1.06)	0.003
neutrophils	977	1.07 (1.05-1.10)	<0.001	466	1.06 (1.03-1.08)	<0.001
CRP	642	1.01 (1.00-1.01)	<0.001	311	1.01 (1.00-1.01)	0.004

IMD: index of multiple deprivation; WCC: white cell count; eGFR: estimated glomerular filtration rate; CRP: C-reactive protein.

In the final model (Table 3.8), short-term mortality was associated with increased age (OR=1.05, 95% CI: 1.03-1.07), decreased duration of hospitalisation after TOX (OR=0.94, 95% CI: 0.91-0.97) and decreased levels of serum albumin (OR=0.83, 95% CI: 0.79-0.88). Considering only toxigenic strains, increased age (OR=1.04, 95% CI: 1.02-1.07), decreased duration of hospitalisation time after TOX (OR=0.94, 95% CI: 0.90-0.97) and decreased levels of serum albumin (OR=0.86, 95% CI: 0.81-0.92) showed an association with short-term mortality.

Table 3.8 Final model of multivariable analysis for short-term mortality

Short-term mortality	All patients (n=487)		Toxigenic-strains (n=269)	
	Odds ratio	p	Odds ratio	p
age	1.05 (1.03 -1.07)	<0.00	1.04 (1.02-1.07)	0.001
albumin	0.83 (0.79-0.88)	<0.00	0.86 (0.81-0.92)	<0.001
disease	0.94 (0.91-0.97)	<0.00	0.94 (0.90-0.97)	0.001

3.5.4.2 Long-term mortality (within 1 year)

After 1 year, more than half of case patients had died (53%, n=132), while 21% (n=57) of control patients and 41% of PCR+ (n=97) and 40% (n=59) of PCR- patients died in the same period. Considering long-term mortality, CDI status, gender, age, disease and pre-test periods, levels of WCC, albumin and CRP and mode of acquisition (Table 3.9) were included in the initial model considering all patients. Toxin, age, gender, disease, pre-test levels of serum WCC, albumin and CRP (Table 3.9) were included in the logistic regression model when considering toxigenic strains for mortality within 1 year as the outcome.

For mortality within 1 year after TOX, increased age (OR=1.04, 95% CI: 1.03-1.06), decreased level of serum albumin (OR=0.92, 95% CI: 0.89-0.95), GDH+/TOX-/PCR+ (OR=2.34, 95% CI: 1.30-4.24) and GDH+/TOX+ (OR=3.04, 95% CI: 1.71-5.41) versus diarrhoea control patients were the variables associated positively with outcome (Table 3.10). Considering only toxigenic strains, increased age (OR=1.04, 95% CI: 1.02-1.06) and decreased levels of serum albumin (OR=0.92, 95% CI: 0.89-0.96) were associated with the outcome (Table 3.10).

Table 3.9 Univariate analysis of long-term mortality

Long-term mortality	All patients			Toxigenic strains		
	n	Odds ratio	p	n	Odds ratio	p
CDI	1,015	2.06 (1.71-2.49)	<0.001	489	1.64 (1.14-2.34)	0.007
gender	1,015	0.93 (0.72-1.20)	0.597	489	0.88 (0.62-1.26)	0.483
age	1,015	1.04 (1.03-1.05)	<0.001	489	1.04 (1.02-1.05)	<0.001
IMD score	931	1.01 (1.00-1.01)	0.084	441	1.01 (1.00-1.02)	0.145
mode of acquisition	1,015	1.40 (1.08-1.81)	0.010	465	1.26 (0.88-1.81)	0.214
CDI severity				109	0.99 (0.44-2.22)	0.985
disease	957	1.01 (1.00-1.01)	0.011	465	1.00 (1.00-1.01)	0.241
pre-test	957	1.02 (1.01-1.03)	<0.001	465	1.01 (1.00-1.02)	0.021
albumin	847	0.91 (0.89-0.93)	<0.001	411	0.90 (0.88-0.93)	<0.001
WCC	997	1.04 (1.02-1.05)	<0.001	481	1.03 (1.01-1.06)	0.004
neutrophils	977	1.04 (1.02-1.06)	<0.001	466	1.03 (1.01-1.06)	0.010
CRP	642	1.00 (1.00-1.01)	0.001	311	1.00 (1.00-1.01)	0.011

IMD: index of multiple deprivation; WCC: white cell count; eGFR: estimated glomerular filtration rate; CRP: C-reactive protein.

Table 3.10 Final model of multivariable analysis for long-term mortality

Long-term mortality	All patients (n=487)		Toxigenic-strains (n=269)	
	Odds ratio	p	Odds ratio	p
GDH-/TOX-[a]	1.00			
GDH+/TOX-/PCR-[b]	1.34 (0.64-2.82)	0.435		
GDH+/TOX-/PCR+[c]	2.34 (1.30-4.24)	0.005		
GDH+/TOX+[d]	3.04 (1.71-5.41)	<0.001		
age	1.04 (1.03-1.06)	<0.001	1.04 (1.03-1.06)	<0.001
albumin	0.92 (0.89-0.95)	<0.001	0.92 (0.88-0.96)	<0.001

[a] diarrhoea control patients [b] non-toxigenic patients [c] toxigenic patients [d] CDI cases

3.5.4.3 Time to death within 1 year

For the Cox regression analysis, CDI status, gender, age, serum levels of WCC and albumin (Table 3.11) were considered in the model for all patients. Presence of toxin, gender, age, serum levels of WCC and albumin (Table 3.11) were considered in the model for only cases and colonised patients. When considering time to death within 1-year, increased age (HR=1.01, 95% CI:

1.00-1.02), increased serum WCC levels (HR=1.02, 95% CI: 1.01-1.04) and decreased serum albumin levels (HR=0.96, 95% CI: 0.94-0.97) had a positive association with the outcome (Table 3.12). For patients infected by toxigenic strains, time to death within 1 year was associated with increased levels of serum WCC (HR=1.03, 95% CI: 1.01-1.05) and decreased levels of serum albumin (HR=0.96, 95% CI: 0.93-0.98) (Table 3.12).

Table 3.11 Univariate analysis of time to death within 1 year

Time to death	**All patients**			**Toxigenic-strains**		
	n	Coef	p	n	Coef	p
CDI status/toxin	345	1.29 (-8.04-10.61)	0.786	229	-5.52 (-30.33-19.29)	0.661
gender	381	-5.82 (-24.98-13.33	0.550	229	-2.22 (-28.83-22.39)	0.859
age	381	-0.69 (-1.29--0.08)	0.026	229	-0.58 (-1.39-0.22)	0.156
IMD score	351	-0.24 (-0.68-0.20)	0.284	207	-0.43 (-0.99-0.13)	0.131
mode of acquisition	371	-5.56 (-25.13-14.01)	0.577	223	2.38 (-22.73-27.49)	0.852
CDI severity				40	-4.79 (-63.63-54.05)	0.870
disease	371	0.13 (-0.08-0.34)	0.217	223	0.25 (-0.13-0.64)	0.193
pre-test	371	-0.05 (-0.39-0.29)	0.776	223	-0.20 (-0.65-0.24)	0.367
albumin	314	3.16 (2.00-4.31)	<0.001	196	3.62 (1.72-5.52)	<0.001
WCC	377	-1.61 (-2.52--0.70)	0.001	227	-1.27 (-2.39--0.16)	0.025
neutrophils	364	-2.27 (-3.46--1.07)	<0.001	218	-1.83 (-3.27-0.39)	0.013
CRP	220	-0.12 (-0.27-0.03)	0.122	139	-0.09 (-0.26-0.08)	0.304

IMD: index of multiple deprivation; WCC: white cell count; eGFR: estimated glomerular filtration rate; CRP: C-reactive protein.

Table 3.12 Final model of survival analysis for time to death within 1 year

Time to death	**All patients (n=344)**		**Toxigenic-strains (n=230)**	
	Haz Ratio	p	Haz Ratio	p
GDH-/TOX-[a]	1.00			
GDH+/TOX-	1.26 (0.82-1.92)	0.290		
GDH+/TOX-	1.26 (0.88-1.81)	0.210		
GDH+/TOX+[d]	1.15 (0.81-1.62)	0.432		
toxin			0.94 (0.70-1.25)	0.656
age	1.01 (1.00-1.02)	0.036		
albumin	0.96 (0.94-0.97)	<0.001	0.96 (0.93-0.98)	<0.001
WCC	1.02 (1.01-1.03)	<0.001	1.02 (1.01-1.04)	<0.001

[a] diarrhoea control patients [b] non-toxigenic patients [c] toxigenic patients [d] CDI cases

3.5.5 CDI severity

Severity was only assessed in the cohort CDI cases and colonised patients as we did not have access to clinical information from the audit patients. Severe disease was present in 44% (n=34) and 23% (n=7) of patients, respectively. In the univariate analysis, presence of toxin, levels of serum WCC, neutrophils and CRP (Table 3.13) were associated with CDI severity. Levels of WCC were not included in the model, as this variable is used for categorising CDI severity. The final logistic regression model (Table 3.14) suggested that there was an association between CDI severity and the presence of the *C. difficile* toxins (OR=3.18, 95% CI: 1.05-9.60).

Table 3.13 Univariate analysis of CDI severity

CDI severity	n	Odds ratio	p
toxin	109	2.65 (1.02-6.87)	0.045
gender	109	1.38 (0.64-3.01)	0.415
age	109	1.00 (0.97-1.02)	0.858
IMD score	100	1.00 (0.98-1.02)	0.918
mode of acquisition	107	0.68 (0.31-1.50)	0.340
disease	107	1.00 (0.98-1.01)	0.676
pre-test	107	0.99 (098-1.01)	0.449
mortality 4 weeks	109	3.44 (0.30-39.13)	0.320
1 year	109	0.99 (0.44-2.22)	0.985
albumin	99	0.98 (0.92-1.04)	0.494
WCC	107	1.14 (1.06-1.23)	<0.001
neutrophils	103	1.16 (1.07-1.27)	<0.001
CRP	87	1.01 (1.00-1.01)	0.052
fidaxomicin	109	0.82 (0.37-1.81)	0.626
metronidazole	109	1.79 (0.74-4.33)	0.195
vancomycin	109	2.19 (0.91-5.31)	0.082
food supplement	109	1.10 (0.34-3.54)	0.875
zopiclone	109	0.38 (0.08-1.91)	0.242
PPI	109	0.48 (0.21-1.12)	0.088
immunosuppressors	109	1.77 (0.53-5.91)	0.352

IMD: index of multiple deprivation; WCC: white cell count; eGFR: estimated glomerular filtration rate; CRP: C-reactive protein; PPI: Proton-pump inhibitor.

Table 3.14 Final model of multivariable analysis for CDI severity

CDI severity	Toxigenic strains (n=87)	
	Odds ratio	p
toxin	3.18 (1.05-9.60)	0.041

3.5.6 Hospitalisation costs

Median hospitalisation costs were higher for case patients (£5,192; IQR: £3,842-7,135), followed by PCR+ (£4,571; IQR: £3,190-6,237), PCR- (£3,842; IQR: £3,109-7,599) and control patients (£3,221; IQR: £2,442-4,558) when outliers were excluded.

Table 3.15 Univariate analysis of hospitalisation costs

Hospitalisation costs	All patients			Toxigenic strains		
	n	expβ	p	n	expβ	p
CDI status toxin	703	1.11 (1.06-1.16)	<0.001	377	1.16 (1.02-1.34)	0.027
gender	787	0.95 (0.85-1.06)	0.381	377	0.91 (0.79-1.04)	0.164
age	787	1.00 (1.00-1.01)	0.004	377	1.00 (1.00-1.01)	0.398
IMD score	770	1.00 (1.00-1.00)	0.962	371	1.00 (1.00-1.00)	0.991
mode of acquisition	774	1.72 (1.55-1.89)	<0.001	376	1.73 (1.54-1.95)	<0.001
CDI severity				85	1.13 (0.83-1.53)	0.442
disease	774	1.02 (1.02-1.02)	<0.001	376	1.02 (1.02-1.02)	<0.001
pre-test	774	1.02 (1.02-1.03)	<0.001	376	1.02 (1.02-1.02)	<0.001
mortality 4 weeks	787	0.99 (0.85-1.16)	0.897	377	0.95 (0.80-1.13)	0.555
mortality 1 year	787	1.24 (1.10-1.38)	0.001	377	1.14 (0.99-1.30)	0.065
albumin	647	0.98 (0.97-0.98)	<0.001	309	0.97 (0.96-0.98)	<0.001
WCC	780	1.00 (0.99-1.01)	0.876	374	1.00 (0.99-1.01)	0.743
neutrophils	767	1.00 (0.99-1.01)	0.559	363	1.00 (1.00-1.01)	0.595
CRP	539	1.00 (1.00-1.00)	0.003	251	1.00 (1.00-1.00)	0.127

IMD: index of multiple deprivation; WCC: white cell count; eGFR: estimated glomerular filtration rate; CRP: C-reactive protein; PPI: Proton-pump inhibitor.

Based on univariate analysis (Table 3.15) and including important variables for the outcome, an initial GLM model with gamma distribution and link log was performed with CDI status, age, gender, disease and pre-test periods, mortality within 1 year, levels of serum albumin and CRP and mode of acquisition as covariates. Increased duration of hospitalisation prior (expβ=1.01, 95% CI: 1.01-1.02) and post (expβ=1.02, 95% CI: 1.01-1.02) TOX and healthcare-associated infection (expβ=1.24, 95% CI: 1.13-1.35) were positively associated with hospitalisation costs (Table 3.16). GDH+/TOX-/PCR- patients were 10% (£5,166±3,374), GDH+/TOX-/PCR+ patients were 18% (£5,531±3,612) and GDH+/TOX+ patients were 25% (£5,848±3,820) more expensive than GDH-/TOX- patients (£4,680±3,057).

Considering only toxigenic strains, toxin, disease and pre-test periods, albumin and mode of acquisition were included in the initial model. In the final model, increase in duration of hospitalisation prior (expβ=1.01, 95% CI: 1.01-1.02) and post (expβ=1.02, 95% CI: 1.01-1.02) and presence of healthcare-associated infection (expβ=1.20, 95% CI: 1.09-1.42) were the variables associated with hospitalisation costs (Table 3.16). The presence of a toxin positive result was not significant to this model and the predicted costs of the model were £5,695±3,585.

Table 3.16 Final model of multivariable analysis for hospitalisation costs

Hospitalisation costs	All patients (n=699)		Toxigenic strains (n=376)	
	expβ	p	expβ	p
GDH-/TOX-[a]	1.00			
GDH+/TOX-/PCR-[b]	1.10 (0.98-1.25)	0.112		
GDH+/TOX-/PCR+[c]	1.18 (1.07-1.31)	0.002		
GDH+/TOX+[d]	1.25 (1.13-1.38)	<0.001		
mode of acquisition	1.24 (1.13-1.35)	<0.001	1.20 (1.09-1.33)	<0.001
disease	1.02 (1.01-1.02)	<0.001	1.02 (1.01-1.02)	<0.001
pre-test	1.01 (1.01-1.02)	<0.001	1.01 (1.01-1.02)	<0.001

[a] diarrhoea control patients [b] non-toxigenic patients [c] toxigenic patients [d] CDI cases

3.5.7 Cost savings with implementation of NAAT assay

FDX has become the first line treatment for CDI in this setting and between 2013 and 2015, 24 non-toxigenic strain patients were unnecessarily treated with FDX (Table 3.17). Considering that the NAAT test (£40 per assay) should have been performed after every GDH+/TOX- result and not excluding duplicates and tests performed more than once in a short period, in the same period, the hospital could have saved £9,640 by not treating any non-toxigenic strain patients.

Table 3.17 Number and costs of GDH+/TOX- results and treatment with fidaxomicin for non-toxigenic strains and non-severe disease

Year	GDH+/TOX- test		Non-toxigenic strain		Non-severe disease	
	Number	Costs	Number	Costs	Number	Costs
2012	127	£5,080	0	£0	0	£0
2013	179	£7,160	1	£1,350	7	£9,450
2014	199	£7,960	15	£20,250	20	£27,000
2015	191	£7,640	8	£10,800	16	£21,600
2016	14	£560	0	£0	0	£0
Total	710	£28,400	24	£32,400	43	£58,050

In 2015 when the test was implemented, 8 of those patients were treated with FDX, indicating a decrease by 50% compared to the previous year but it still shows that unnecessary treatment has been given to some patients. This amount can increase if we consider that non-severe CDI patients were also treated with FDX, which is not recommended by PHE.

3.6 Discussion

GDH+/TOX-/PCR- had on average worse clinical outcomes compared to GDH+/TOX-/PCR+ patients. The latter group showed significantly lower mortality rates during hospitalisation and within 1 year, CDI severity and hospitalisation costs compared with CDI cases. Similar results were found in studies conducted in the UK and US (Planche et al., 2013, Polage et al., 2012, Polage et al., 2015). In the multivariate analysis, no differences related to CDI status were observed in all-cause mortality within 4 weeks after TOX. However, GDH+/TOX-/PCR+ were 2.3 times and CDI cases were 3.0 times more likely to die within 1 year after the test compared to diarrhoea control patients. Long-term mortality rate was considered in this thesis but of course this may be more related to comorbidities than to the infection by *C. difficile*. It also actually highlights that patients with comorbidities are more susceptible to *C. difficile* infection.

Comparing only patients infected by a toxigenic strain, the presence of toxin was not significant in the model, in contrast to a study conducted in 4 UK hospitals that suggested a poor outcome related to the presence of toxin (Planche et al., 2013). Time to discharge was similar in the GDH+/TOX-/PCR+

and GDH+/TOX+ groups and lower compared to GDH+/TOX-/PCR- by 0.7, 0.8 and 0.6 times when compared to control patients. Hospitalisation costs were 18% and 25% more expensive for GDH+/TOX-/PCR+ and CDI cases respectively, compared to control patients. CDI severity was the only clinical outcome where the presence of a positive result for toxin test was significant compared to those patients who only presented with the gene for toxigenic strain. In this case, toxin positive patients were 3.2 times more likely to present with severe disease.

Ribotype profile was similar in GDH+/TOX-/PCR+ and CDI case groups and matched England's profile from 2008 to 2015 that showed the emergence of the RT078, RT002, RT005, RT014/020 and RT015 strains (PHE, 2016). Moreover, they were similar to the European profile in 2008 when RT014/020 and RT078 were the most commonly found strains in 34 countries, while the UK was experiencing an outbreak with RT027 (Bauer et al., 2011). This epidemic strain was present in only 1.6% of CDI cases and 4.9% of toxigenic patients during the period of this study.

Although UK Department of Health guidelines currently advise that GDH+/TOX- patients are unlikely to have CDI and are therefore not subject to mandatory reporting (Department of Health, 2012a), in our setting GDH+/TOX- results were predominantly caused by toxigenic strains. These patients carrying a toxigenic strain constitute a clinically significant segment of individuals as they encompass an important reservoir and potential source of CDI transmission. Symptomatic toxigenic patients often display dysbiosis and are likely to shed a high load of *C. difficile* in their stools, thus contributing to increased skin and environmental contamination when compared to

asymptomatic colonised individuals (Alasmari et al., 2014, Sethi et al., 2010). Studies have suggested potential transmission from symptomatic (Planche et al., 2013, Mawer et al., 2017) and asymptomatic (Blixt et al., 2017, Curry et al., 2013, Longtin et al., 2016) toxigenic patients in different countries. An aggravating risk factor is that symptomatic patients usually stay longer in hospital waiting for the right diagnosis and adequate treatment. Thus, preventive measures are indicated for those patients to avoid transmission and new cases (Planche et al., 2013, Mawer et al., 2017, Longtin et al., 2016) but the decision to treat these patients should be based on individual clinical assessment (Planche et al., 2013).

The combination of GDH detection, TOX and molecular tests appears to be a good diagnostic option, as it can overcome all limitations of each test performed alone, and it can also confirm the identification of the toxigenic isolates. However, this test is more complex, needs more laboratory equipment to be performed and it is about five to ten times more expensive (Planche et al., 2013). Although some researchers support the use of only NAAT for diagnosis of CDI because of its high NPV, it may not be performed alone as it is not specific for CDI, not distinguishing disease from colonisation and it may thus be responsible for over-diagnosis (Planche et al., 2013, Polage et al., 2015). An ultrasensitive assay for detection and quantification of toxins has been developed in the US as a new tool that would have not only the potential to overcome the limitations of current diagnostics but would also have prognostic value identifying severe patients that need more attention. New studies have been undertaken to improve, optimise and refine the assay (Song et al., 2015, Pollock, 2016).

Of those 237 toxigenic isolates identified, 131 (55%) received CDI treatment with FDX, VAN or MTZ. NAAT assay was implemented at RLBUHT in 2015, when 84% of toxigenic samples were treated as a CDI case and in the first month of 2016, 75% of samples were treated in the same way. However, 24 non-toxigenic isolates were over treated with FDX, corresponding to £32,400 of unnecessary spending. It is known that more *C. difficile* tests are done in the UK than in other European countries (Bauer et al., 2011), but even if a NAAT test were performed for all 569 GDH+/TOX- result, this would have saved £9,640 between 2013 and 2015. Furthermore, given the PHE recommendations for CDI treatment, 30 CDI cases and 13 toxigenic isolates were also over treated with FDX as they presented with mild or moderate CDI, resulting in an additional overspend of £58,050.

The study does have some limitations. Ninety-nine patients were excluded as the PCR result was not available to categorise them. It was only possible to derive CDI severity for cohort patients as all clinical information was collected by the nurses and was available in the patients' CRFs. Medical records of audit patients were not accessed, and all information was collected from electronic databases (iPM, ICE and EPMA). Clinical outcomes considered in this study were not always related to CDI; cause of death was not accessed during the study, and thus it is not possible to affirm when the bacterial infection was directly or indirectly responsible for deaths especially since the patients in this study were mainly elderly and had known comorbidities, LoS was longer than usual and sometimes related to chronic and serious conditions; also, costs of hospitalisation do not represent the real cost of the patients as they were based on HRG codes and national tariff values. Lastly, the study did not cover

a large period after the implementation of NAAT assay, and thus meaningful comparison, before and after, of treatment given to patients cannot be made.

In summary, GDH+/TOX- should be treated appropriately as we show that in our setting, more than 60% were infected by a toxigenic strain. The 3-step algorithm appears to be the best screening option, as it combines the identification of toxin positive patients as well as *C. difficile* producers of toxins. This combination may be able to prevent transmission, avoid unnecessary treatment and thereby prevent unnecessary healthcare and resource spending.

Chapter 4

Procalcitonin as a screening test for early stratification and prognosis of hospitalised patients infected by *Clostridium difficile*.

4.1 Introduction

Procalcitonin (PCT) is a biomarker of 114 to 116 amino acids, and is the precursor of the hormone calcitonin (Davies, 2015, Meisner, 2010). It is an immune modulator produced by the thyroid, and can be induced by severe systemic inflammation, its production being activated in all parenchymal tissues (Meisner, 2014). PCT can be used for the diagnosis of severe bacterial infections and sepsis, evaluation of the severity of infection, evaluation of the course of disease and indication and follow up of antibiotic therapy, including in helping make clinical decisions to stop antibiotics (Meisner, 2014, Meisner, 2010).

In the last decade, PCT has gained increasing acceptance with many studies published comparing the use of PCT with CRP for diagnostic and prognostic evaluation of diseases (Liu et al., 2015, Meisner, 2010). Although PCT has been employed in many countries in Europe, it has not been tested routinely in the UK yet. In the local hospital, the current tests used include CRP and WCC.

For CDI, only four studies have been published comparing levels of PCT and CDI severity (Dazley et al., 2015, Rao et al., 2013), diagnosis and PCR positivity (Shapiro et al., 2017, Popiel et al., 2015). High levels of PCT were associated with CDI severity in both studies, but no association was found with other outcomes. Thus, because of the limited analysis conducted so far with CDI, we have undertaken this study to assess the performance of PCT as a screening test for early stratification and determining the prognosis of hospitalised patients infected by *C. difficile*.

4.2 Patients and methods

4.2.1 Patient cohort

The cohort of patients from both phases I and II of our CDI studies were included in this PCT study. They have been fully described in Chapter 2. Covariates used in this chapter were described in section 2.2.3.

4.2.2 PCT measurement

Salvaged serum samples collected during recruitment, follow-up 1 (around 2 weeks after recruitment) and follow-up 2 (4 to 6 weeks after recruitment) were used to measure PCT levels retrospectively. All samples were stored at -80°C. PCT was measured by an eletrochemiluminescence immunoassay (ELECYSIS BRAHMS kit) at LCL, RLBUHT, which is a clinically accredited laboratory. Samples were measured regardless of the date of collection (same as recruitment date).

According to the manufacturer, the sensitivity of assay is $\leq$0.02ng/mL and intra and inter-assay variation ranges from 1 to 9% and 3 to 16%, respectively. Also, sensitivity was 96%, specificity 66%, positive predictive value (PPV) 78% and NPV 93% when considering the cut-off value of 0.5ng/mL. The assay takes 18 minutes in the laboratory (Roche Diagnostics and Cobas, 2009). In general, healthy patients have PCT levels below 0.05ng/mL, levels above 0.5ng/mL suggest the possibility of a systematic infection, while severe sepsis is associated with levels between 2 to 10ng/mL (ThermoFischer Scientific, 2017).

4.2.3 Clinical outcomes

CDI diagnosis, time to discharge, short-term mortality (within 4 weeks) and long-term mortality (within 1 year), time to death within 1 year, CDI severity and CDI recurrence were assessed in this study. The definitions of the clinical outcomes have been described in section 2.2.4, and in previous publications on this cohort of patients (Swale et al., 2014b, Swale et al., 2014a).

4.3 Statistical analysis

4.3.1 Patient characteristics

PCT and CRP results are initially presented as median and IQR by groups according to diagnostic tests (GDH, TOX and PCR). Results above reference range were also presented for both measurements. For the analysis, clinical information and outcomes were presented as median and interquartile range (IQR), or frequency (F) and percentage (P) for continuous and categorical variables, respectively, by TOX results. T-test and ANOVA were employed to compare normally distributed continuous variables and Mann-Whitney U test or Kruskal Wallis to compared non-normally distributed variables between all groups and groups categorised according to TOX, respectively, and chi-square test to compare categorical variables.

4.3.2 Multivariable analysis

A univariate analysis was performed for every clinical outcome to identify potential covariates for the multivariable analysis. All statistically significant

variables were included in the initial model and variables were excluded one by one according to the highest p-value until all remained covariates were significant. A new model with remaining variables was performed after each exclusion and statistical significance was set at $p < 0.05$.

Two models were utilised to assess the potential association of PCT and CRP with each outcome, and thus these variables were included in all models regardless of the p-value. Logistic regression was used to assess CDI diagnosis, mortality within 4 weeks and 1 year, CDI severity and CDI recurrence. Multivariable Cox proportional hazards analysis was performed for time to discharge and time to death within 1 year, no censoring was applied for time to discharge but patients who did not die within 1 year after diagnosis were censored.

As the day of blood sample collection for PCT and CRP measurements was not standardised, a sensitivity analysis was performed in both models including patients by time from toxin test to PCT or CRP measurement. PCT and CRP kinetic graphs published in the literature (Póvoa, 2002, ThermoFischer Scientific, 2017) were used to identify the period when biomarkers were elevated in bacterial infections. Considering that PCT levels in general start decreasing around day 3 after the infection and CRP levels between days 3 to 7 after the infection, patients were included in the analysis when tests were performed within 2 to 5 days after the toxin test and CRP results were included when the test was performed within 0 to 8 days. Additionally, one analysis was undertaken including all samples regardless of the time point at which PCT or CRP tests were performed. All statistical

analyses were performed using STATA version 14.0 (StataCorp LP, College Station, Texas).

4.4 Results

4.4.1 PCT results in CDI patients

1,042 samples from 715 patients were tested. Patients were recruited between 2008 and 2015 and during this period, *C. difficile* clinical diagnostic methods changed twice, and so it was not possible to group all patients accurately. The levels of PCT and CRP in patients with different diagnostic patterns are shown in Table 4.1. The median levels of serum PCT were on average similar in CDI cases (0.22ng/L, IQR: 0.09-0.58), GDH+/TOX-/PCR- (0.21ng/L, IQR: 0.08-0.48) and GDH+/TOX-/PCR+ (0.20ng/L, IQR: 0.09-0.34) patients. All groups showed a decrease in PCT levels during follow-up at 2 weeks (follow-up 1) and 4 to 6 weeks (follow-up 2). TOX- patients had the same median value at baseline and follow-up 1, but only 8 patients were included in the 2-week follow-up.

Conversely, analysis of CRP levels (Table 4.1) showed that the levels were on average higher in CDI cases (85mg/L, IQR: 39-183) and GDH+/TOX-/PCR+ (62mg/L, IQR: 22-115) than in GDH+/TOX-/PCR- (36mg/L, IQR: 16-74), GDH-/TOX- (32mg/L, IQR: 12-123) and TOX- (38mg/L, IQR: 9-124) patients.

As both variables were skewed (Figure 4.1), they were log transformed (log_{10}) for the multivariable analysis. A summary of CRP and PCT values log_{10} transformed at baseline and PCT at follow-ups 1 and 2 are shown in Figure

4.2 and the comparison between all groups and CDI cases are shown in Table 4.2.

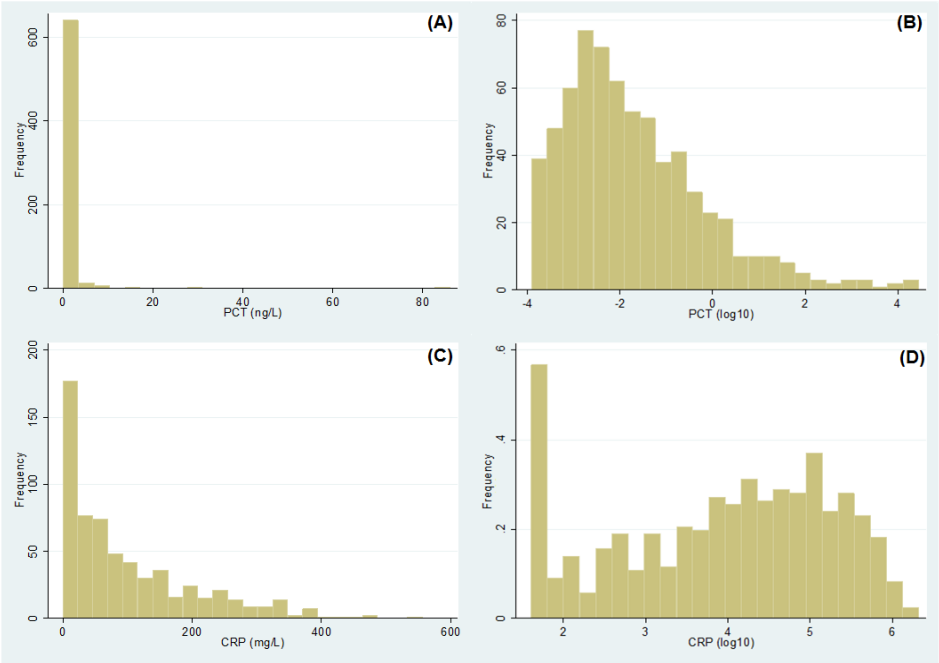

Figure 4.1 (A) Histogram of PCT absolute values (B) Histogram of log transformed PCT values (C) Histogram of CRP absolute values (D) Histogram of log transformed CRP values.

Table 4.1 PCT and CRP results by groups and time points

	Healthy control		**TOX-[a]**		**GDH-/TOX-[b]**		**GDH+/TOX-PCR-[c]**		**GDH+/TOX-PCR+[d]**		**GDH+/TOX+[e]**	
	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)
PCT baseline (ng/L)	74	0.03 (0.02-0.05)	188	0.11 (0.06-0.32)	57	0.12 (0.06-0.34)	16	0.21 (0.08-0.48)	35	0.20 (0.09-0.34)	304	0.22 (0.09-0.58)
PCT follow-up 1 (ng/L)	0	.	8	0.11 (0.09-0.17)	31	0.07 (0.04-0.19)	9	0.06 (0.04-0.20)	23	0.08 (0.05-0.16)	61	0.10 (0.06-0.27)
PCT follow-up 2 (ng/L)	64	0.04 (0.03-0.05)	36	0.05 (0.03-0.06)	46	0.05 (0.04-0.08)	5	0.12 (0.04-0.18)	20	0.06 (0.04-0.09)	65	0.09 (0.05-0.19)
CRP baseline (mg/L)	0	.	189	38 (9-124)	61	32 (12-123)	13	36 (16-74)	37	62 (22-115)	321	85 (39-183)

TOX: toxin test, GDH: glutamate dehydrogenase test, PCR: polymerase chain reaction.
[a] diarrhoea control patients tested before 2012. [b] diarrhoea control patients. [c] diarrhoea control patients colonized by a *C. difficile* non-toxigenic strain. [d] carrier patients colonized by a *C. difficile* toxigenic strain. [e] CDI cases.

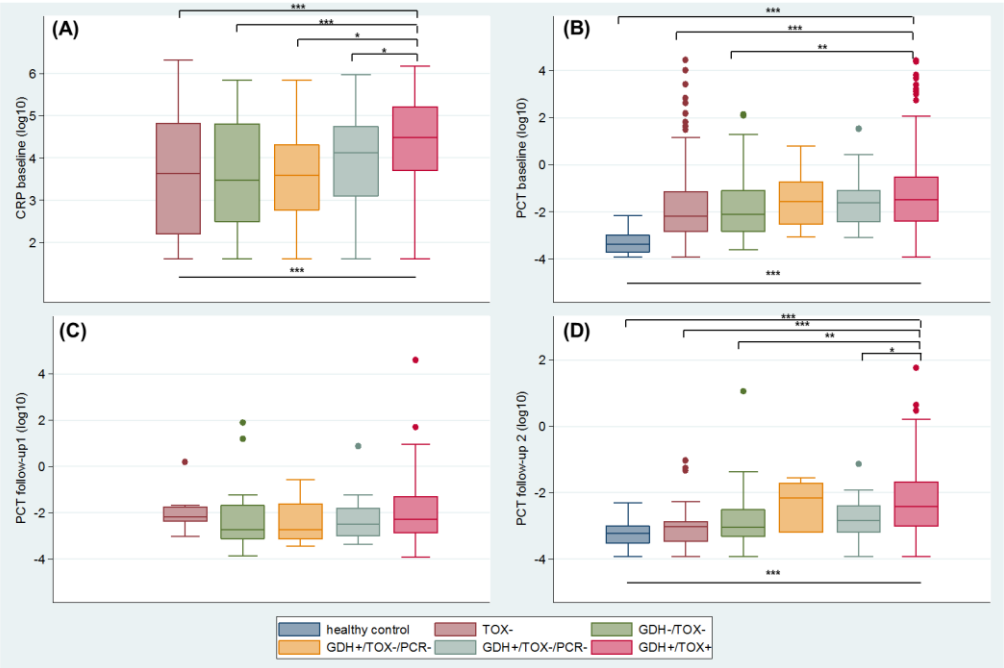

Figure 4.2 (A) CRP baseline (log_{10}) results by group, (B) PCT baseline (log_{10}) results by group, (C) PCT follow-up 1 (log_{10}) results by group, (D) PCT follow-up 2 (log_{10}) results by group.

CDI case patients had CRP values (log_{10}) higher than all groups while PCT values (log_{10}) in CDI cases at baseline were only higher compared to the healthy control group, the group not tested for GDH but TOX- and the GDH-/TOX- group. Four to 6 weeks after recruitment, the CDI case group had similar PCT values (log_{10}) compared to the non-toxigenic group (GDH+/TOX-/PCR-) but higher compared to all others.

For further analyses, patients were categorised according to the toxin test result (TOX- and TOX+) and healthy control patients were excluded. A summary of log_{10} transformed values of baseline CRP and PCT and follow-up 1 and follow-up 2 PCT is shown in Figure 4.3. Patient characteristics are presented in Table 4.3.

Table 4.2 Comparison between p-value of CDI case and others groups

	all groups	healthy control	TOX-[a]	GDH TOX-[b]	GDH+ TOX- PCR-[c]	GDH+ TOX- PCR+[d]
	p	p	p	p	p	p
PCT baseline (log_{10})	<0.001	<0.001	<0.001	0.009	0.455	0.166
PCT follow-up 1 (log_{10})	0.517	.	0.865	0.153	0.360	0.359
PCT follow-up 2 (log_{10})	<0.001	<0.001	0.001	0.004	0.826	0.050
CRP baseline (log_{10})	<0.001	.	<0.001	<0.001	0.014	0.042

TOX: toxin test, GDH: glutamate dehydrogenase test, PCR: polymerase chain reaction. [a] diarrhoea control patients tested before 2012. [b] diarrhoea control patients. [c] diarrhoea control patients colonized by a *C. difficile* non-toxigenic strain. [d] carrier patients colonized by a *C. difficile* toxigenic strain.

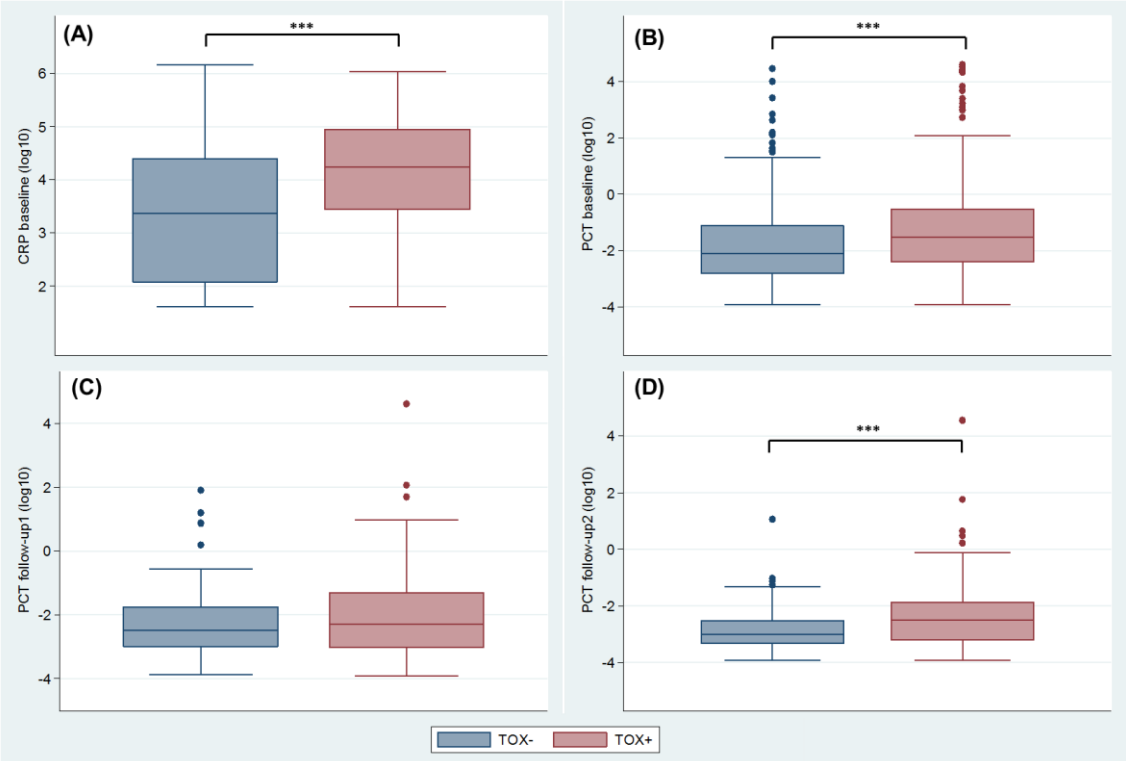

Figure 4.3 (A) CRP baseline (log_{10}) results by toxin test, (B) PCT baseline (log_{10}) results by toxin test, (C) PCT follow-up 1 (log_{10}) results by toxin test, (D) PCT follow-up 2 (log_{10}) results by toxin test.

The median age of TOX+ group was higher (73 vs 64 years, p<0.001) and median Los was twice as high as the diarrhoea control patients (22 vs 11 days,

p<0.001). 42% (n=141) of TOX+ patients and 21% of TOX- (n=8) patients presented with severe disease as categorised in section 2.2.4. Mortality was also higher during hospitalisation [7% (n=23) vs 3% (n=9), p=0.023] and within 1 year after the toxin test [31% (n=104) vs 15% (n=45), p<0.001]. These patients also presented with leucocytosis (WCC>11×10^9cells/L) and hypoalbuminemia (albumin<35g/L).

The median CRP levels were twice as high in the CDI cases compared to the TOX- patients [88 (IQR: 40-183) vs 36 (IQR: 10-114) mg/L]. CRP levels were above the reference range for all patients, apart from 2 samples, while PCT levels were higher in CDI cases [0.22 (IQR: 0.09-0.59) vs 0.12 (IQR=0.06-0.33) ng/mL). 103 of 334 (31%) of CDI case samples and 59 of 291 (20%) of diarrhoea control samples had levels higher than 0.5ng/mL which may indicate a potential systemic infection (Meisner, 2014). When log transformed, this difference was significant for both CRP (p<0.001) and PCT (p<0.001) levels. Moreover, 93% (n=313) of patients who tested positive for TOX were treated with either FDX, VAN or MTZ.

Table 4.3 Patients characteristics by toxin result

	TOX-[a] (n=305)		**TOX+[b] (n=335)**		
	n	Median(IQR) F (P)	n	Median(IQR) F (P)	p
Demographics					
Age (years), median (IQR)	305	64 (48-74)	335	73 (59-80)	<0.001
gender (female, %)	305	169 (55.4)	335	185 (55.2)	0.962
IMD score (score), median (IQR)	276	37.3 (18.5-57.8)	266	33.2 (17.3-55.8)	0.317
Clinical					
CCI (score), median (IQR)	305	1 (0-2)	335	1 (0-2)	<0.001
mode of acquisition (HCAI, %)	305	147 (48.2)	335	199 (59.4)	0.063
CDI severity (%)	38	8 (21.0)	335	141 (42.1)	0.026
CDI recurrence (%)	0		335	55 (16.4)	
Hospitalisation					
Study phase (phase II, %)	305	166 (54.4)	334	78 (23.3)	<0.001
LoS (days), median (IQR)	292	11 (6-24)	334	22 (11-44)	<0.001
disease (days), median (IQR)	282	7 (4-15)	334	13 (7-26)	<0.001
pre-test (days), median (IQR)	282	3 (1-9)	334	5 (1-17)	<0.001
Time to death (days), median (IQR)	86	336.5 (127-700)	168	234.5 (75.5-615.5)	0.307
Mortality hospitalisation (%)	305	9 (3.0)	335	23 (6.9)	0.023
4 weeks (%)	305	8 (2.6)	335	18 (5.4)	0.078
1 year (%)	305	45 (14.8)	335	104 (31.0)	<0.001

Table 4.3 (continued) Patients' characteristics by toxin result

	TOX-[a] (n=305)		TOX+[b] (n=335)		
	n	Median(IQR) F (P)	n	Median(IQR) F (P)	p
Laboratory results, median (IQR)					
WCC baseline (10^9/L)	299	8.8 (6.4-12.5)	334	11.4 (7.9-17.1)	<0.001
albumin baseline (g/L)	285	34 (26-39)	328	29 (24-34)	0.018
CRP baseline (mg/L)	294	35.5 (10-114)	327	88 (40-183)	<0.001
(log_{10})	294	3.6 (2.3-4.7)	325	4.5 (3.7-5.2)	<0.001
(>5 mg/L)	294	35.5 (10-114)	325	89 (40-183)	<0.001
time to test (days)	221	0 (-1-2)	301	0 (-1-2)	0.901
PCT baseline (ng/mL)	291	0.12 (0.06-0.33)	334	0.22 (0.09-0.59)	0.092
(log_{10})	291	-2.1 (-2.81--1.12)	334	-1.5 (-2.4--0.5)	<0.001
(>0.5 ng/mL)	59	1.36 (0.72-3.23)	103	1.16 (0.74-3.26)	0.442
time to test (days)	288	3 (2-4)	365	3 (3-5)	<0.001
follow-up 1 (ng/mL)	71	0.08 (0.05-0.17)	81	0.10 (0.05-0.27)	0.169
(log_{10})	71	-2.5 (-3--1.75)	81	-2.30 (-3.02--1.31)	0.115
follow-up 2 (ng/mL)	107	0.05 (0.04-0.08)	82	0.08 (0.04-0.16)	0.201
(log_{10})	107	-3.0 (-3.32--2.51)	82	-2.5 (-3.2--1.9)	0.001
Medicines (%)					
CDI treatment[c]	305	56 (18.4)	335	313 (93.4)	<0.001
Fidaxomicin	305	26 (8.5)	335	51 (15.2)	0.009
Vancomycin	305	23 (7.5)	335	250 (74.6)	<0.001
Metronidazole	305	18 (5.9)	335	135 (40.3)	<0.001

TOX: toxin test, IMD: index of multiple deprivation, CCI: Charlson comorbidity index, LoS: length of stay, WCC: white cell count, CRP: C-reactive protein, PCT: procalcitonin.

[a] diarrhoea control patients, [b] CDI case patients, [c] treatment with any antibiotic (standard treatment or fidaxomicin)

4.4.2 Multivariable analyses

4.4.2.1 CDI diagnosis

Initially, a univariate analysis (Table 4.4) was performed to guide the inclusion of covariates for the multivariable models. Thus, the initial model included age, pre-test period, Charlson comorbidity index (CCI) score, concentration of serum albumin, WCC, PCT and CRP.

Table 4.4 Univariate analysis of CDI diagnosis as clinical outcome

variables	n	Odds Ratio	95% CI	p
age	640	1.03	1.02-1.034	<0.001
gender (female)	640	0.99	0.73-1.36	0.962
IMD score 2015	542	1.00	0.99-1.00	0.316
CCI 2011	640	1.28	1.14-1.43	<0.001
mode of acquisition	616	1.35	0.98-1.86	0.064
pre-test	616	1.02	1.01-1.03	0.001
albumin	613	0.99	0.97-1.00	0.018
WCC	633	1.06	1.03-1.08	<0.001
PCT (log_{10})	625	1.26	1.13-1.41	<0.001
CRP (log_{10})	619	1.58	1.39-1.81	<0.001

A relationship between PCT levels and the presence of *C. difficile* toxins was found when patients tested within 2 days of the toxin test were included (OR=1.63, 95% CI: 1.04-2.58) (Table 4.5). This association did not remain when more time points were included in the model. Increased age also showed an association with diagnosis in this model. Increased levels of WCC were associated in all other models, increased CCI score showed association when patients were tested within 4 and 5 days after the toxin test and at all time points after the toxin test. Increased pre-test period was a covariate when patients were tested within 5 days after the toxin test and all time points were

included. The results of all multivariable analysis for each clinical outcome and time point are presented in the Appendix (3-107).

Table 4.5 Multivariable and sensitivity analysis of PCT (log_{10}) as covariate and CDI diagnosis as clinical outcome

PCT[a]	n	Odds Ratio	95% CI	p	covariates
<2	64	1.76	1.04-2.58	0.035	age
<3	226	1.19	0.98-1.44	0.075	age, WCC
<4	358	1.13	0.97-1.32	0.110	age, CCI, WCC
<5	444	1.12	0.98-1.29	0.089	age, pre-test, CCI, WCC
all	557	1.12	0.99-1.27	0.075	age, pre-test, CCI, WCC

[a] time point: time between toxin test and PCT test

Levels of CRP were associated with CDI diagnosis when patients were tested within 3 (OR=1.29, 95% CI: 1.08-1.53), 4 (OR=1.32, 95% CI: 1.12-1.56), 5 (OR=1.33, 95% CI: 1.13-1.56), 6 (OR=1.35-95% CI: 1.15-1.59), 7 (OR=1.35-95% CI: 1.14-1.58), and 8 (OR=1.34, 95% CI: 1.14-1.58) days after the toxin test and all time points (OR=1.35-95% CI: 1.16-1.57) were included (Table 4.6). An increased pre-test period and increased levels of WCC also showed associations in all these models. Increased CCI score was associated with the outcome when patients were tested within 3 days after the toxin test and all time points were included. There was also a relationship between increased age and CDI diagnosis when all time points were included in the model.

Table 4.6 Multivariable analysis and sensitivity of CRP (log_{10}) as covariate and CDI diagnosis as clinical outcome

CRP[a]	n	Odds Ratio	95% CI	p	covariates
<0	168	1.16	0.89-1.53	0.271	pre-test
<1	287	1.13	0.91-1.39	0.277	pre-test, WCC
<2	353	1.18	0.98-1.42	0.072	pre-test, WCC
<3	414	1.29	1.08-1.53	0.005	pre-test, CCI, WCC
<4	445	1.32	1.12-1.56	0.001	pre-test, WCC
<5	471	1.33	1.13-1.56	0.001	pre-test, WCC
<6	476	1.35	1.14-1.58	<0.001	pre-test, WCC
<7	477	1.35	1.15-1.59	<0.001	pre-test, WCC
<8	478	1.34	1.14-1.58	<0.001	pre-test, WCC
all	542	1.35	1.16-1.57	<0.001	age, pre-test, CCI, WCC

[a] time point: time between toxin test and CRP test

4.4.2.2 Time to discharge

In the initial model, toxin, age, mode of acquisition, recruitment phase (when patients were recruited), serum levels of albumin, CRP and PCT were included according to the univariate analysis (Table 4.7).

Table 4.7 Univariate analysis of time to discharge as clinical outcome

variable	n	Coef	95% CI	p
toxin	626	17.23	10.78-23.67	<0.001
age	638	0.19	0.01-0.37	0.038
gender (female)	638	-4.36	-10.88-(-2.17)	0.190
IMD score 2015	547	0.07	-0.07-0.21	0.344
CCI 2011	638	-0.09	-2.20-2.02	0.933
mode of acquisition	628	29.70	23.48-35.91	<0.001
albumin	616	-0.51	-0.75-(-0.26)	<0.001
WCC	635	0.07	-0.21-0.34	0.631
PCT baseline (log_{10})	598	2.60	0.36-4.84	0.023
PCT follow-up 1 (log_{10})	130	7.53	0.78-14.28	0.029
PCT follow-up 2 (log_{10})	158	13.03	7.32-18.74	<0.001
CRP (log_{10})	620	4.34	1.81-6.88	0.001
Recruitment phase	638	-11.54	-18.25-(-4.83)	0.001

Increased levels of PCT were associated with increased risk of delayed discharge when considering patients tested within 3 (HR=0.87, 95% CI: 0.80-0.95) and 5 (HR=0.93, 95% CI: 0.87-0.99) days after the toxin test and all time points (HR=0.93, 95% CI: 0.88-0.99) (Table 4.8). Considering these 3 models, covariates positively associated with time to discharge were decreased age and CAI; toxin negative test, decreased age, increased levels of albumin and CAI; and toxin negative test, increased levels of albumin and CAI, respectively. Measurement of follow-up 1 and 2 were not included in the models because of the low number of samples tested.

Table 4.8 Multivariable and sensitivity analysis of PCT (log_{10}) as covariate and time to discharge as clinical outcome

PCT[a]	**n**	**Haz ratio**	**95% CI**	**p**	**covariates**
<2	64	1.07	0.88-1.31	0.480	mode of acquisition
<3	225	0.87	0.80-0.95	0.002	age, mode of acquisition
<4	354	0.93	0.87-1.00	0.061	toxin, age, mode of acquisition
<5	440	0.93	0.87-0.99	0.028	toxin, age, albumin, mode of acquisition
all	548	0.93	0.88-0.99	0.029	toxin, albumin, mode of acquisition

[a] time point: time between toxin test and PCT test

Serum levels of CRP had no association with time to discharge in any scenario. However, toxin negative test, CAI and increased levels of serum albumin decreased the risk of delayed discharge, as observed in the models below (Table 4.9).

Table 4.9 Multivariable and sensitivity analysis of CRP (log_{10}) as covariate and time to discharge as clinical outcome

CRP[a]	n	Haz ratio	95% CI	p	covariates
<0	168	0.93	0.81-1.07	0.307	toxin, mode of acquisition
<1	287	0.98	0.89-1.09	0.746	toxin, albumin, mode of acquisition
<2	353	0.98	0.90-1.07	0.627	toxin, albumin, mode of acquisition
<3	411	0.94	0.87-1.02	0.152	toxin, mode of acquisition
<4	440	0.96	0.88-1.04	0.275	toxin, albumin, mode of acquisition
<5	466	0.93	0.86-1.01	0.071	toxin, albumin, mode of acquisition
<6	471	0.93	0.86-1.01	0.096	toxin, albumin, mode of acquisition
<7	472	0.94	0.86-1.01	0.100	toxin, albumin, mode of acquisition
<8	473	0.94	0.86-1.01	0.098	toxin, albumin, mode of acquisition
all	534	0.94	0.86-1.01	0.098	toxin, albumin, mode of acquisition

[a] time point: time between toxin test and CRP test

4.4.2.3 Mortality rates

4.4.2.3.1 Short-term mortality (within 4 weeks)

Initial models included the presence of toxin, age, CCI, mode of acquisition, recruitment phase), serum levels of albumin, PCT and CRP. Covariates were chosen after univariate analysis (Table 4.10).

Table 4.10 Univariate analysis of short-term mortality as clinical outcome

Mortality 4 weeks	n	Odds ratio	95% CI	p
toxin	640	2.11	0.90-4.92	0.085
age	652	1.03	1.00-1.06	0.020
gender (female)	652	0.94	0.43-2.07	0.886
IMD score 2015	551	1.00	0.98-1.02	0.924
CCI 2011	652	1.38	1.14-1.67	0.001
mode of acquisition	628	2.69	1.07-6.80	0.036
LoS	638	1.00	0.98-1.01	0.599
disease	628	0.99	0.97-1.01	0.353
pre-test	628	1.00	0.98-1.02	0.824
albumin	625	0.97	0.94-1.00	0.022
WCC	645	1.01	0.98-1.03	0.589
PCT baseline (log_{10})	612	1.30	1.04-1.61	0.019
PCT follow-up 1 (log_{10})	132	0.15	0.00-7.07	0.332
PCT follow-up 2 (log_{10})	172	1.38	0.29-6.62	0.689
CRP (log_{10})	631	1.48	1.04-2.11	0.031
Recruitment phase	652	0.49	0.19-1.24	0.130

Table 4.11 shows that serum levels of PCT were only statistically significant after inclusion of patients who were tested within 4 (OR=1.33, 95% CI: 1.00-1.76) and 5 (OR=1.30, 95% CI: 1.02-1.66) days after TOX, and at all time points (OR=1.28, 95% CI: 1.01-1.62). In the first model, no covariates were included, but increased CCI was associated in the last two models and HCAI were also associated with mortality within 4 weeks in the last model.

Table 4.11 Multivariable and sensitivity analysis of PCT (log_{10}) as covariate and short-term mortality as clinical outcome

PCT[a]	n	Odds ratio	95% CI	p	covariates
<2	64	1.69	0.64-4.48	0.291	CCI
<3	226	1.32	0.96-1.81	0.086	CCI
<4	358	1.33	1.00-1.76	0.048	.
<5	444	1.30	1.02-1.66	0.033	CCI
all	557	1.28	1.01-1.62	0.038	CCI, mode of acquisition

[a] time point: time between toxin test and PCT test

Peak levels of serum CRP were only associated with mortality within 4 weeks when patients who were tested on the same day or before the toxin test (OR=1.01, 95% CI: 1.00-1.01) were included. No covariates were included in the model (Table 4.12), but HCAI and increased CCI score were related to mortality within 4 weeks in other models.

Table 4.12 Multivariable and sensitivity analysis of CRP (log_{10}) as covariate and short-term mortality as clinical outcome

CRP[a]	n	Odds ratio	95% CI	p	covariates
<0	168	3.03	1.09-8.44	0.034	.
<1	287	1.85	0.95-3.62	0.073	.
<2	353	1.66	0.91-3.02	0.097	mode of acquisition
<3	414	1.42	0.90-2.24	0.128	mode of acquisition
<4	445	1.40	0.92-2.12	0.116	CCI, mode of acquisition
<5	471	1.35	0.91-2.01	0.136	CCI, mode of acquisition
<6	476	1.35	0.91-2.01	0.137	CCI, mode of acquisition
<7	477	1.35	0.91-2.00	0.139	CCI, mode of acquisition
<8	478	1.35	0.91-2.01	0.136	CCI, mode of acquisition
all	544	1.42	0.96-2.10	0.079	CCI, mode of acquisition

[a] time point: time between toxin test and CRP test

4.4.2.3.2 Long-term mortality (within 1 year)

When mortality within 1 year was the outcome, toxin, age, CCI, LoS, mode of acquisition, recruitment phase, levels of albumin, PCT and CRP were included in the initial multivariable model (Table 4.13).

Table 4.13 Univariate analysis of long-term mortality as clinical outcome

Mortality 1 year	**n**	**Odds ratio**	**95% CI**	**p**
toxin	640	2.60	1.76-3.85	<0.001
age	652	1.03	1.02-1.05	<0.001
gender (female)	652	0.87	0.61-1.26	0.465
IMD score 2015	551	1.00	0.99-1.01	0.818
CCI 2011	652	1.60	1.42-1.81	<0.001
mode of acquisition	628	1.67	1.14-2.45	0.008
LoS	638	1.00	1.00-1.01	0.025
disease	628	1.00	1.00-1.01	0.092
pre-test	628	1.01	1.00-1.02	0.056
albumin	625	0.98	0.97-0.99	0.002
WCC	645	1.01	1.00-1.03	0.112
PCT baseline (log_{10})	612	1.25	1.11-1.41	<0.001
PCT follow-up 1 (log_{10})	132	1.35	1.03-1.77	0.029
PCT follow-up 2 (log_{10})	172	2.31	1.52-3.51	<0.001
CRP (log_{10})	631	1.27	1.09-1.48	0.002
Recruitment phase	652	0.90	0.62-1.31	0.581

Mortality within 1 year was related to increased levels of PCT (Table 4.14), increased CCI score and decreased levels of serum albumin when considering patients tested within 3 days (OR=1.40, 95% CI: 0.85-2.29) after toxin test, to increased level of PCT, increased age, increase CCI score and HCAI when considering patients tested within 4 (OR=1.33, 95% CI: 1.12-1.58) and 5 days (OR=1.28, 95% CI: 1.10-1.48) after toxin test and all time points (OR=1.22-95% CI: 1.06-1.40).

Table 4.14 Multivariable and sensitivity analysis of PCT (log_{10}) as covariate and long-term mortality as clinical outcome

PCT[a]	**n**	**Odds ratio**	**95% CI**	**p**	**covariates**
<2	64	1.40	0.85-2.29	0.188	CCI
<3	226	1.43	1.15-1.77	0.001	CCI, albumin
<4	358	1.33	1.12-1.58	0.001	age, CCI, mode of acquisition
<5	444	1.28	1.10-1.48	0.002	age, CCI, mode of acquisition
all	557	1.22	1.06-1.40	0.005	age, CCI, mode of acquisition

[a] time point: time between toxin test and PCT test

Increased levels of serum CRP (Table 4.15), increased age and increased CCI score were associated with mortality within 1 year considering patients tested within 1 (OR=1.33, 95% CI: 1.01-1.75), 2 (OR=1.41, 95% CI: 1.11-1.80) and 3 (OR=1.27-95% CI: 1.03-1.56) days after toxin test. Also, HCAI was associated with the outcome in the last model.

Table 4.15 Multivariable and sensitivity analysis of CRP (log_{10}) as covariate and long-term mortality as clinical outcome

CRP[a]	n	Odds ratio	95% CI	p	covariates
<0	168	1.32	0.92-1.90	0.136	age, CCI
<1	287	1.33	1.01-1.75	0.040	age, CCI
<2	353	1.41	1.11-1.80	0.005	age, CCI
<3	414	1.27	1.03-1.56	0.026	age, CCI, mode of acquisition
<4	445	1.22	1.00-1.48	0.055	age, CCI, mode of acquisition
<5	471	1.15	0.95-1.39	0.141	age, CCI, mode of acquisition
<6	476	1.17	0.97-1.41	0.094	age, CCI, mode of acquisition
<7	477	1.17	0.97-1.41	0.097	age, CCI, mode of acquisition
<8	478	1.16	0.97-1.40	0.110	age, CCI, mode of acquisition
all	544	1.18	0.99-1.40	0.066	age, CCI

[a] time point: time between toxin test and CRP test

4.4.2.3.3 Time to death within 1 year

The same variables of mortality within 1 year were included in the initial model, as all variables were not significant in the univariate analysis (Table 4.16): toxin, age, CCI, LoS, mode of acquisition, recruitment phase, levels of serum albumin, PCT and CRP.

Table 4.16 Univariate analysis of time to death within 1 year as clinical outcome

Time to death	n	Coef	95% CI	p
toxin	149	-15.79	-49.97-18.39	0.363
age	152	-0.45	-1.53-0.64	0.417
gender (female)	152	17.69	-14.06-49.43	0.273
IMD score 2015	142	0.02	-0.74-0.78	0.956
CCI 2011	152	0.02	-8.64-8.68	0.997
mode of acquisition	151	-18.58	-52.15-14.99	0.276
LoS	151	-0.12	-0.49-0.26	0.540
disease	151	-0.101	-0.66-0.44	0.698
pre-test	151	-0.28	-1.05-0.48	0.464
albumin	145	0.44	-0.80-1.68	0.485
WCC	152	-0.47	-1.34-0.39	0.281
PCT baseline (log_{10})	139	-9.03	-19.98-1.92	0.105
PCT follow-up 1 (log_{10})	28	-14.24	-32.63-4.14	0.123
PCT follow-up 2 (log_{10})	28	-8.18	-46.71-30.36	0.666
CRP (log_{10})	146	-13.18	-26.76-0.39	0.057
Recruitment phase	152	8.31	-24.91-41.53	0.622

Levels of serum PCT were not associated with time to death within 1 year (Table 4.17), however, peak of serum CRP were positively associated (Table 4.18) in almost all time points. Considering patients tested within 1 (HR=1.29, 95% CI: 1.01-1.64) or 2 (HR=1.34, 95% CI: 1.09-1.65) days after toxin test any other variable was included. Between 4 and 8 days after test and including all time points, increased CCI was a covariate in all models and the only one when considering patients tested within 4 days (HR=1.26, 95% CI: 1.05-1.50). HCAI was significant when patients were tested within 3 days after toxin test (HR=1.00, 95% CI: 1.00-1.00). Also, phase II patients were more likely to be dead within 1 year when patients were tested within 5 (HR=1.00, 95% CI: 1.00-1.00), 6 (HR=1.23, 95% CI: 1.05-1.44), 7 (HR=1.23, 95% CI: 1.05-1.44), 8 (HR=1.23, 95% CI: 1.05-1.44) days after toxin test and at all time points (HR=1.20, 95% CI: 1.03-1.40).

Table 4.17 Multivariable and sensitivity analysis of PCT (log_{10}) as covariate and time to death within 1 year as clinical outcome

PCT[a]	n	Haz ratio	95% CI	p	covariates
<2	28	1.26	0.82-1.94	0.295	.
<3	93	1.14	0.98-1.32	0.083	CCI
<4	142	1.14	1.00-1.30	0.056	CCI
<5	180	1.11	0.99-1.25	0.068	recruitment phase, CCI
all	229	1.09	0.98-1.21	0.126	recruitment phase, CCI

[a] time point: time between toxin test and PCT test

Table 4.18 Multivariable and sensitivity analysis of CRP (log_{10}) as covariate and time to death within 1 year as clinical outcome

CRP[a]	n	Haz ratio	95% CI	p	covariates
<0	68	1.37	0.98-1.92	0.064	.
<1	119	1.29	1.01-1.64	0.040	.
<2	144	1.34	1.09-1.65	0.005	.
<3	175	1.26	1.05-1.50	0.011	recruitment phase, mode of acquisition
<4	196	1.26	1.06-1.49	0.009	CCI
<5	209	1.22	1.04-1.44	0.014	recruitment phase, CCI
<6	212	1.23	1.05-1.44	0.011	recruitment phase, CCI
<7	212	1.23	1.05-1.44	0.011	recruitment phase, CCI
<8	213	1.23	1.05-1.44	0.012	recruitment phase, CCI
all	223	1.20	1.03-1.40	0.018	recruitment phase, CCI

[a] time point: time between toxin test and CRP test

4.4.2.4 CDI severity

Toxin, age, gender, mode of acquisition, recruitment phase, levels of serum albumin, PCT and CRP were included in the initial model (Table 4.19). Level of serum WCC was part of categorization of CDI severity and not included in the multivariable analysis.

Table 4.19 Univariate analysis of CDI severity as clinical outcome

CDI severity	n	Odds ratio	95% CI	p
toxin	367	2.60	1.09-6.17	0.031
age	367	0.99	0.98-1.01	0.331
gender (female)	367	1.08	0.71-1.65	0.711
IMD score 2015	292	1.00	0.99-1.01	0.678
CCI 2011	367	0.97	0.85-1.10	0.608
mode of acquisition	365	0.73	0.48-1.12	0.154
LoS	365	1.00	1.00-1.00	0.783
disease	365	1.00	1.00-1.01	0.604
pre-test	365	0.99	0.98-1.00	0.156
Mortality 4 weeks	367	0.41	0.13-1.26	0.119
Mortality 1 year	367	0.68	0.43-1.08	0.099
albumin	357	1.00	0.98-1.01	0.662
WCC	365	1.14	1.10-1.19	<0.001
PCT baseline (log_{10})	339	1.32	1.13-1.56	<0.001
PCT follow-up 1 (log_{10})	84	1.00	0.76-1.33	0.979
PCT follow-up 2 (log_{10})	85	1.30	0.86-1.97	0.212
CRP (log_{10})	356	1.45	1.18-1.77	<0.001
Recruitment phase	367	0.83	0.53-1.31	0.435

CDI severity was associated with the level of serum PCT (Table 4.20) when considering patients tested within 3 (OR=1.56, 95% CI: 1.18-2.07), 4 (OR=1.33, 95% CI: 1.09-1.63) and 5 (OR=1.22, 95% CI: 1.03-1.45) days and at all time points (OR=1.30, 95% CI: 1.11-1.52).

Table 4.20 Multivariable and sensitivity analysis of PCT (log_{10}) as covariate and CDI severity as clinical outcome

PCT[a]	n	Odds ratio	95% CI	p	covariates
<2	34	1.57	0.86-2.87	0.143	.
<3	118	1.56	1.18-2.07	0.002	.
<4	191	1.33	1.09-1.63	0.006	.
<5	250	1.22	1.03-1.45	0.020	.
all	328	1.30	1.11-1.52	0.001	.

[a] time point: time between toxin test and PCT test

Levels of serum CRP were also associated (Table 4.21) with CDI severity when patients tested within 1 (OR=1.44, 95% CI: 1.07-1.93), 2 (OR=1.53, 95% CI: 1.17-1.99), 3 (OR=1.59, 95% CI: 1.23-2.05), 4 (OR=1.54, 95% CI: 1.21-1.96), 5 (OR=1.54, 95% CI: 1.22-1.94), 6 (OR=1.54, 95% CI: 1.22-1.94), 7 (OR=1.54, 95% CI: 1.22-1.94) and 8 days (OR=1.52, 95% CI: 1.21-1.92) after toxin test and all time points (OR=1.40, 95% CI:1.13-1.73) were included. Any covariate was included in the models.

Table 4.21 Multivariable and sensitivity analysis of CRP (log_{10}) as covariate and CDI severity as clinical outcome

CRP[a]	n	Odds ratio	95% CI	p	covariates
<0	100	1.29	0.84-1.98	0.245	.
<1	181	1.44	1.07-1.93	0.017	.
<2	222	1.53	1.17-1.99	0.002	.
<3	259	1.59	1.23-2.05	<0.001	.
<4	279	1.54	1.21-1.96	<0.001	.
<5	294	1.54	1.22-1.94	<0.001	.
<6	296	1.54	1.22-1.94	<0.001	.
<7	296	1.54	1.22-1.94	<0.001	.
<8	297	1.52	1.21-1.92	<0.001	.
all	321	1.40	1.13-1.73	0.002	.

[a] time point: time between toxin test and PCT test

4.4.2.5 CDI recurrence

In the initial model, age, gender, mortality within 1 year, LoS, recruitment phase and serum levels of PCT and CRP were included as independent variables (Table 4.22).

Table 4.22 Univariate analysis of CDI recurrence as clinical outcome

CDI recurrence	n	Odds ratio	95% CI	p
toxin	.	.	.	.
age	367	1.04	1.02-1.07	<0.001
gender (female)	367	1.24	0.69-2.22	0.475
IMD score 2015	292	1.00	0.98-1.01	0.522
CCI 2011	292	0.92	0.77-1.11	0.399
mode of acquisition	365	0.87	0.49-1.56	0.645
LoS	365	1.01	1.00-1.02	<0.001
disease	365	1.02	1.01-1.02	<0.001
pre-test	365	1.01	1.00-1.02	0.232
Mortality 1 year	367	3.68	2.04-6.64	<0.001
albumin	357	0.99	0.97-1.02	0.564
WCC	365	1.00	0.99-1.02	0.569
PCT baseline (log_{10})	363	1.00	0.82-1.21	0.962
PCT follow-up 1 (log_{10})	104	0.53	0.22-1.30	0.165
PCT follow-up 2 (log_{10})	102	1.52	1.00-2.29	0.048
CRP (log_{10})	356	1.17	0.90-1.53	0.236
Recruitment phase	397	0.27	0.12-0.59	0.001

The only variable that showed association with recurrence rate was increased age. No relationship between serum levels of PCT or CRP (Tables 4.23 and 4.24) and CDI recurrence was found in this cohort.

Table 4.23 Multivariable and sensitivity analysis of PCT (log_{10}) as covariate and CDI recurrence as clinical outcome

PCT[a]	n	Odds ratio	95% CI	p	covariates
<2	34	0.80	0.33-1.94	0.620	.
<3	118	0.94	0.55-1.59	0.806	age
<4	191	1.21	0.89-1.66	0.225	age
<5	250	1.06	0.81-1.39	0.647	age
all	328	1.01	0.81-1.26	0.960	age

[a] time point: time between toxin test and PCT test

Table 4.24 Multivariable and sensitivity analysis of CRP (log_{10}) as covariate and CDI recurrence as clinical outcome

CRP[a]	n	Odds ratio	95% CI	p	covariates
<0	100	1.47	0.65-3.32	0.360	age
<1	181	1.13	0.73-1.75	0.596	age
<2	222	1.20	0.83-1.72	0.333	age
<3	259	1.30	0.91-1.86	0.145	age
<4	279	1.28	0.91-1.80	0.153	age
<5	294	1.22	0.88-1.69	0.229	age
<6	296	1.24	0.90-1.72	0.185	age
<7	296	1.24	0.90-1.72	0.185	age
<8	297	1.25	0.90-1.73	0.180	age
all	321	1.10	0.82-1.48	0.505	age

[a] time point: time between toxin test and CRP test

4.5 Discussion

CRP is the most recognized and widely used biomarker for monitoring bacterial infection, but it can also be elevated in viral infections and is a traditional biomarker of inflammation (Vikse et al., 2015). CRP starts rising after 12 to 24 hours, reaches a peak around 20 to 72 hours after the stimulus and remains elevated for 3 to 7 days (Schneider and Lam, 2007, Vikse et al., 2015, Simon et al., 2004). As a non-specific biomarker, in our cohort only two patients had CRP levels within the reference range. One study in intensive care unit patients found that CRP levels remained high (80mg/L) at the end of antibiotic therapy (Deliberato et al., 2013). Moreover, 3 patients with an unfavourable outcome in a pneumonia study (de Jager et al., 2009) had elevated PCT levels while CRP levels were decreasing during the 7 day period.

PCT is an accurate biomarker for severe bacterial infection and sepsis (Simon et al., 2004, Nargis et al., 2014). After 2 to 4 hours of infection, levels of PCT

start rising, peak after 24 to 48 hours and start decreasing around 2 to 3 days when response to antibiotic treatment is effective (Vikse et al., 2015, Banerjee et al., 2002, ThermoFischer Scientific, 2017). The half-life of PCT is 22 to 30 hours (Lee, 2013, Jin and Khan, 2010), but its levels can persist until completely recovery (Lee, 2013).

Considering that PCT levels can remain elevated until day 3 while CRP can remain elevated until day 7, PCT levels were positively associated with CDI diagnosis, time to discharge, long-term mortality and CDI severity while CRP levels were positively associated with time to death within 1 year and CDI severity. It is important to highlight that associations were observed at some time points when other clinical outcomes were analysed. When considering absolute values, PCT levels were higher in the CDI case group, but no difference was found between groups. On the other hand, CRP levels were higher in CDI cases than in controls, however, and above the reference range in almost all patients. Similar results were previously found in a study of 50 patients in Israel (Shapiro et al., 2017). Also, no difference in PCT levels were found comparing TOX-/PCR+ and TOX-/PCR- in a Canadian study with 64 subjects (Popiel et al., 2015). In our multivariable analysis, results were not consistent for CRP as it was associated with CDI diagnosis only when we included patients tested 3 days after the toxin test.

Although time to discharge was negatively associated with PCT levels in our study, this association was not found in a previous study with CDI patients (Rao et al., 2013), also, NICE guidance (NICE, 2015b) concluded based on 7 studies that PCT measurement may not be a reliable method to assess LoS as it is multifactorial and highly influenced by local policy and clinicians'

preferences. In studies with 101 (Stolz et al., 2009), 81 (Deliberato et al., 2013) and 58 (Annane et al., 2013) patients with pneumonia and sepsis, no differences were observed between groups comparing control and PCT-based algorithm groups.

PCT was also related to long-term mortality in our study. Short-term mortality showed an association in some scenarios when more patients were included. In cancer patients with bacterial infection, PCT levels showed an association with mortality during the study, but no association was found for CRP and WCC levels (Murat Sedef et al., 2016). Prognosis of mortality for septic patients is still unclear, but two studies with 86 (Meng et al., 2009) and 54 patients (Jain et al., 2014) showed an association with elevated levels of PCT during admission and mortality within 4 weeks (Jain et al., 2014).

CDI severity has previously been associated with PCT levels (Rao et al., 2013, Dazley et al., 2015). The definition of severity was different in each study and TOX+ patients were included when PCT levels were drawn within 24 hours (Dazley et al., 2015), and with TOX+ and TOX-/PCR+ patients with PCT measurement carried out between 24 to 72 hours (Rao et al., 2013). Also, CDI could be identified as severe when PCT was higher than 0.2 ng/mL (Rao et al., 2013) and 0.5 ng/mL (Dazley et al., 2015). PCT is also associated with severity from other bacterial infections including pneumonia (de Jager et al., 2009, Kim et al., 2013, Don et al., 2007), cellulitis (Noh et al., 2016), paediatric bacterial meningitis (Hu et al., 2015) and pyelonephritis (Park et al., 2013).

The PCT assay is not considered an expensive test and economic evaluation studies have concluded that the use of the new biomarker could decrease

costs of hospitalisation (Ito et al., 2017, Stojanovic et al., 2017, Schuetz et al., 2015, Balk et al., 2017) and could be cost-effective when used to guide antibiotic therapy for acute respiratory tract infections (Michaelidis et al., 2014), sepsis (Westwood et al., 2015, Harrison and Collins, 2015) and meningococcal disease (Bell et al., 2015).

Our study has several limitations. Firstly, PCT measurement was conducted retrospectively. We assumed that all the process of sampling and storage conditions did not influence the stability of our aliquots. As the samples were collected during patient recruitment, the time between *C. difficile* testing and recruitment was variable and in some cases, it took longer than 3 days. Also, the day of *C. difficile* test may not be the day when symptoms started nor even when the patient was infected. A sensitivity analysis was conducted to include more patients and also assess a potential association between timings. While the study was being undertaken over an 8 year period, the diagnostic method and algorithm changed twice, and thus some patients could not be properly categorised. Furthermore, our cohort was recruited in two different phases, the first during an epidemic phase (2008-2012) after the 2006-2007 outbreak, tended to be more severe with higher mortality and recurrence rates. The second cohort was recruited during an endemic phase (2013-2015) when the number of cases reached a plateau. The medical diagnosis of diarrhoea control patients was not exhaustively assessed in this study and another bacterial infection cannot be excluded.

In summary, this study has identified several potential new associations between serum PCT and clinical outcomes in CDI patients. Further studies with PCT measured at the same time as CDI diagnosis, or after the initial

symptoms, are required. Furthermore, a randomised controlled trial would be required to determine whether the measurement of PCT represented a clinically effective and cost-effective test in the clinical management of CDI.

Chapter 5

Interventions for *Clostridium difficile* Infection: A Systematic Review of Economic Evaluations

5.1 Introduction

C. difficile strains have acquired resistance to many antibiotics and disinfectants, further contributing to high transmissibility and contamination (CDC, 2013). Infection significantly increases morbidity, mortality and is associated with excess hospital stays. Moreover, CDI remains endemic in several hospitals and continues to be a major problem for healthcare systems (NICE, 2015a).

A recent systematic survey (Lytvyn et al., 2016) found five published guidelines for the prevention of CDI: Health Protection Agency/Department of Health (Department of Health & Health Protection Agency, 2008), European Society for Clinical Microbiology and Infectious Disease (Vonberg et al., 2008), American College of Gastroenterology (Surawicz et al., 2013), Association of Professionals in Infection Control and Epidemiology (APIC, 2013), and Society for Healthcare Epidemiology of America/Infectious Diseases to Society of America (Dubberke et al., 2014). Strategies to control and prevent the infection involve mainly two approaches: (1) a reduction in the environmental burden of *C. difficile* and minimisation of its spread (deep cleaning and disinfection of environment, personnel hygiene, use of protective clothing and containment methods), and (2) rationalisation of antibiotics use (implementation of stewardship programs, monitor antimicrobial resistance). The most recent guideline (Dubberke et al., 2014) also recommends implementation of a laboratory-based alert system for notification of new cases to healthcare professionals, active surveillance and reporting of CDI data, education of healthcare staff, environmental service personnel, hospital administration, patients with CDI and their families, and measurement of compliance with

Center for Disease Control and Prevention (CDC) or World Health Organization (WHO) recommendations.

In the UK, several initiatives have been implemented in hospitals since 2003 to decrease the levels of exposure and the risk of recurrent infections (Marufu et al., 2015, Hughes et al., 2013). However, there remain uncertainties surrounding their effectiveness, and the optimal use of healthcare resources. The NICE guidance on the use of FDX from 2012 (NICE, 2012), for instance, focuses on safety and efficacy, and not its cost-effectiveness, though the All Wales Medicines Strategy Group (AWMSG) and Scottish Medicines Consortium (SMC) have recently published appraisals recommending the use of FDX for treatment of CDI patients with first recurrence (AWMSG, 2012, SMC, 2012) or severe disease (AWMSG, 2012), based on economic evidence.

This chapter aims to review the economic evidence in order to highlight current evidence on the cost-effectiveness of strategies to limit *C. difficile* spread and infection, and to identify methodological limitations.

5.2 Methods

The protocol of this study has been registered with the International Prospective Register of Systematic Reviews (PROSPERO) under registration number CRD42016024893. The study is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (Moher et al., 2009).

5.2.1 Eligibility criteria

The review considered published, original economic evaluations of interventions for the prevention, management or treatment of CDI in healthcare settings. Pre-clinical studies, reviews, commentaries and conference abstracts were excluded from our analysis. There was no language restriction.

5.2.2 Search strategy

A systematic search was performed in January 2016 using 6 different databases: MEDLINE and Embase (via OVID), EconLit (via EBSCO), and the Database of Abstracts of Reviews of Effects, NHS Economic Evaluation database and Health Technology Assessment database (each via CRD).

The search strategy (appendix 106) included terms related to CDI and economics, according to keywords utilised in previously published reviews (Glanville et al., 2009, Nanwa et al., 2015).

5.2.3 Study selection

Two reviewers worked independently to conduct the searches and verify the studies. Titles and abstracts were initially screened for eligibility, full-texts were screened when potential relevant studies reporting interventions for CDI were identified. Full economic evaluations were included if they compared both the costs and consequences of two or more strategies (NICHSR, 2003).

5.2.4 Data extraction

The following study characteristics were extracted: year of publication, country of publication, nature of the intervention, comparators, type of study, type of economic evaluation, costing perspective, time horizon, currency and cost year. The following study results were extracted: mean total costs for each intervention and outcomes, expressed as quality-adjusted life years (QALYs), disability-adjusted life years (DALYs) or other, according to the study.

5.2.5 Study adherence to the CHEERS statement

We used the Consolidated Health Economic Evaluation Reporting Standards (CHEERS) statement guidelines (Husereau et al., 2013) to assess the quality of reporting of included studies. Specific attention was paid to items concerning methodology: target population and subgroups, setting and location, study perspective, comparators, time horizon, discount rate, choice of health outcomes, measurement of effectiveness, measurement and valuation of preference based outcomes, estimating resources and costs, currency, price date, and conversion, choice of model, assumptions, and analytical methods.

5.3 Results

5.3.1 Study selection

Our search identified 2,229 unique studies of which the majority (2,200) were excluded after title and abstract screening as they were not specific to the species *Clostridium difficile* or did not assess interventional measures (Figure 5.1). After full text screening, 18 further studies were excluded for not reporting a full economic evaluation, leaving 11 studies for inclusion in our systematic review.

5.3.2 Study characteristics

Studies mainly focused on treatment, including FDX (Bartsch et al., 2013, Stranges et al., 2013, Konijeti et al., 2014, Nathwani et al., 2014, Wagner et al., 2014, Marković, 2014) and faecal microbiota transplant (FMT) (Varier et al., 2014, Varier et al., 2015, Konijeti et al., 2014). Other studies considered the cost-effectiveness of probiotics (Allen et al., 2013), vaccination of at-risk patients (Lee et al., 2010), and screening hospital admission (Bartsch et al., 2012a). All identified studies were published between 2010 and 2015. The majority were from US (Lee et al., 2010, Bartsch et al., 2012a, Bartsch et al., 2013, Stranges et al., 2013, Konijeti et al., 2014, Varier et al., 2014, Varier et al., 2015), followed by UK (Allen et al., 2013, Nathwani et al., 2014) and a single study each from Canada (Wagner et al., 2014) and Serbia (Marković, 2014).

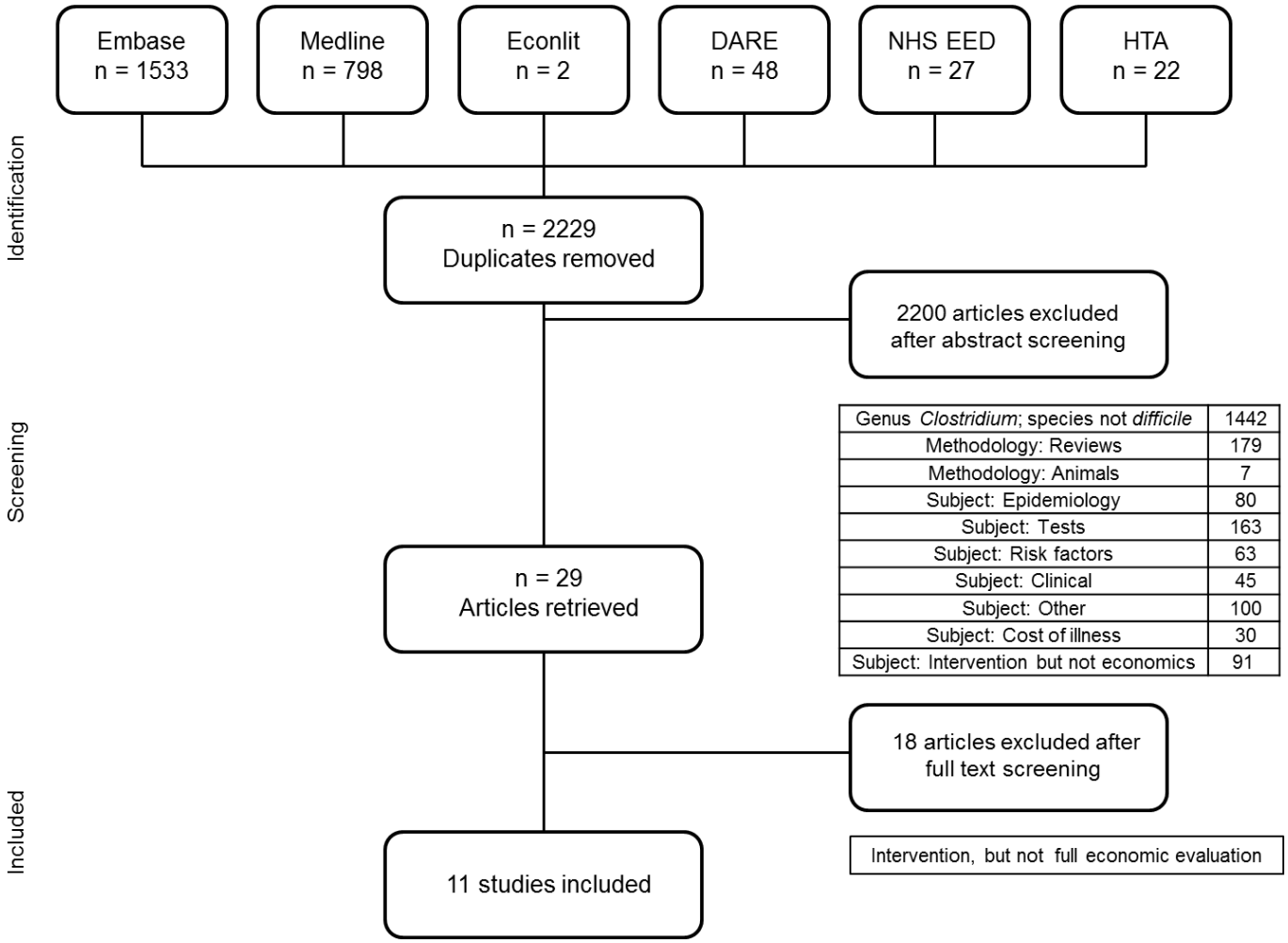

Figure 5.1 Flowchart of study selection process

Characteristics relating to the methods of each study are presented in Table 5.1. All studies were cost-effectiveness (CEA) or cost utility analysis (CUA), 10 were based on decision analytical models and 1 was a clinical trial. A third-party payer perspective was used in 6 studies from the US (Lee et al., 2010, Bartsch et al., 2012a, Bartsch et al., 2013, Stranges et al., 2013, Varier et al., 2014, Varier et al., 2015), healthcare system perspective in 4 studies from the UK, Canada and Serbia (Allen et al., 2013, Nathwani et al., 2014, Wagner et al., 2014, Marković, 2014), hospital perspective in 2 studies from the US (Bartsch et al., 2012a, Lee et al., 2010) and societal perspective in 2 studies from the US (Lee et al., 2010, Konijeti et al., 2014). Economic outcomes considered included cost per QALY gained (Bartsch et al., 2012a, Bartsch et al., 2013, Allen et al., 2013, Konijeti et al., 2014, Nathwani et al., 2014, Varier et al., 2014, Varier et al., 2015, Stranges et al., 2013), cost per DALY averted (Lee et al., 2010), cost per recurrence avoided (Wagner et al., 2014), cost per life-year saved (Marković, 2014), and cost per avoided colectomy (Marković, 2014).

Table 5.1 Characteristics of economic evaluation studies included in the systematic review

Study	Country	Context	Comparator	Type of study	Type of evaluation	Perspective view	Time horizon	Currency and year	CHEERS
Lee et al, 2010	US	Vaccines	(1) vaccines for patients at risk (2) vaccines for CDI patients to prevent recurrences	Decision analytic model	CEA	Societal, hospital and third-party payers	N/R	2009 US dollars	11/14
Bartsch et al, 2012	US	Screening admissions	PCR assay	Decision analytic model	CEA	Hospital and third-party payers	N/R	2011 US dollars	9/14
Allen et al, 2013	UK	Prevention	Placebo and Probiotics	multicentre, randomised, double-blind, placebo-controlled, parallel-arm trial	CUA	Healthcare provider	1 year	2011 GBP	9/14
Bartsch et al, 2013	US	Treatment	(1) M and V (2) F for all (3) F based on strain type	Decision analytic model	CEA	Third-party payer	N/R	2012 US dollars	11/14
Stranges et al, 2013	US	Treatment	V and F	Decision analytic model	CUA	Third-party payer	23 years	2011 US dollars	11/14

Table 5.1 (continued) Characteristics of economic evaluation studies included in the systematic review

Study	Country	Context	Comparator	Type of study	Type of evaluation	Perspective view	Time horizon	Currency and year	CHEERS
Konijeti et al, 2014	US	Treatment for recurrence	(1) M, V, F and FMT via colonoscopy (2) M, V, F and FMT via duodenal infusion (3) M, V, F and FMT via enema (4) M, V, F and FMT all via (5) M, V and F	Decision analytic model	CEA	Societal	1 year	2012 US dollars	11/14
Nathwani et al, 2014	UK	Treatment for severe disease and recurrence	(1) V and F for severe CDI (2) V and F for first recurrence	Decision analytic model	CEA	Healthcare provider	1 year	2010/2011 GBP	12/14
Varier et al, 2014	US	Treatment for recurrence	(1) M and FMT (2) V and FMT	Decision analytic model	CEA	Third-party payer	90 days	2011 US dollars	12/14

Table 5.1 (continued) Characteristics of economic evaluation studies included in the systematic review

Study	Country	Context	Comparator	Type of study	Type of evaluation	Perspective view	Time horizon	Currency and year	CHEERS
Wagner et al, 2014	Canada	Treatment for severe disease and recurrence	(1) V and F for severe CDI (2) V and F for first recurrence	Decision analytic model	CEA	Healthcare provider	60 days	US dollars	10/14
Markovic et al, 2014	Serbia	Treatment	V and F	Decision analytic model	CEA	Healthcare provider	90 days	Republic of Servia dinars (RSD)	12/14
Varier et al, 2015	US	Treatment for recurrence	V and FMT	Decision analytic model	CEA	Third-party payer	90 days	2011 US dollars	13/14

US: United States, UK: United Kingdom, PCR: Polymerase chain reaction, M: Metronidazole, V: Vancomycin, F: Fidaxomicin, FMT: faecal microbiota transplant, CEA: cost-effectiveness analysis, CUA: cost-utility analysis, GBP: British pounds, N/R: Not reported

5.3.3 Quality of reporting

The methods and quality of reporting are summarised in Table 5.1. The quality of reporting was generally high. Study population was described in four studies (Lee et al., 2010, Nathwani et al., 2014, Wagner et al., 2014, Allen et al., 2013). Setting and location was reported in just one study (Allen et al., 2013). Three studies (Bartsch et al., 2012a, Bartsch et al., 2013, Lee et al., 2010) did not mention time horizon of the model and two studies (Marković, 2014, Wagner et al., 2014) did not specify the year adopted for costs of interventions. Only one study (Allen et al., 2013) used a preference-based method for estimate utility scores. All other items were reported in all selected studies however not always with a full description, justification or information of model choices being given explicitly.

5.3.4 Results of individual studies

The results of selected studies were summarised in the Table 5.2 according to the interventional measure addressed in the studies.

Table 5.2 Results of economic evaluation studies included in the systematic review

Study	Costs	Additional information	QALY/DALY	ICER	Cost-effective intervention
Lee et al, 2010	Costs per vaccine: $100 to $1,600	Vaccine efficacy model: 25 to 100% *C. difficile* risk model: 0.1 to 25%	DALYs by infection status[a]: 1st recurrence: -0.0029 2nd recurrence: -0.0043 CDI: -0.0014	< $80,412/DALY prevented for all rates of vaccine efficacy when $25 and risk >2.5% $50 and risk >10% y $100 and risk >15% cost < $400	Depends on *C. difficile* risk, vaccine costs and efficacies. Cost-effective on different scenarios.
Bartsch et al, 2012	Screening (per test[a]): $7.66 Gloves (per pair[a]): $0.0861 Gown: $0.922 Technician wage (per hour[a]): $17.96 Nurse wage (per hour[a]): $31.10	*C. difficile* colonization on admission model: 0.5 to 20% Contact isolation compliance model: 25 to 75%	QALYs by severity[a]: Mild CDI: 0.88 Severe CDI: 0.817 Colectomy: 0.536	≤ $256/QALY on all scenarios	Screening
Allen et al, 2013	Cost per patient[b]: Pl: £8,010 Pr: £8,420	N/A	QALYs gains[b]: Pr - Pl: <0.0014	£189,662/QALY	Placebo

Table 5.2 (continued) Results of economic evaluation studies included in the systematic review

Study	Costs	Additional information	QALY/DALY	ICER	Cost-effective intervention
Bartsch et al, 2013	Cost per treatment[a]: M: 585 V: $1,032 F: $3,360	N/A	QALYs by severity[a]: Non severe CDI: 0.88 Severe CDI: 0.817	(1) >$8.8 million/QALY (2) dominated (3) >$43.7 million/QALY	Given the current costs of fidaxomicin, it is not cost-effective
Stranges et al, 2013	Cost per patient[b]: V: $12,306 F: $13,422	N/A	QALYs per patient[b]: V: 16.551 F: 16.568	$67,576/QALY	F
Konijeti et al, 2014	Cost per patient[b]: M: $3,941 V: $2,912 – 3,531 F: $4,261 – 4,628 FMT: $3,149 – 4,208	N/A	QALYs by group[b]: M: 0.8292 V: 0.8484 – 0.8580 F: 0.8596 – 0.8653 FMT: 0.8543 – 0.8719	(1) V x FMT colonoscopy $17,016/QALY (2) V x FMT via duodenal infusion $97,352/QALY (3) V x FMT $17,016/QALY (4) V x F $184,023/QALY	FMT via colonoscopy
Nathwani et al, 2014	Costs per patient[b]: (1) V: £571 F: £2,567 (2) V: £800 F: £3,630	N/A	QALYs by group[b]: (1) V: 0.705 F: 0.715 (2) V: 0.692 F:0.711	(1) £16,529/QALY (2) -£21,079/QALY	F in both cases

Table 5.2 (continued) Results of economic evaluation studies included in the systematic review

Study	Costs	Additional information	QALY/DALY	ICER	Cost-effective intervention
Varier et al, 2014	Cost per patient[b]: M: $1,167 V: $1,890 FMT: $1,669	N/A	QALYs by group[b]: M: 0.238 V: 0.241 FMT: 0.242	(1) $124,964/QALY (2) dominant	(1) M (2) FMT
Wagner et al, 2014	Total costs for 1000 patients[b]: (1) V: $8,866,593 F: $10,677,167 (2) V: $9,056,376 F: $11,119,038	n of patients with recurrence/1000 patients[b] V: 230 F: 93 n of patients experiencing second recurrence/1000 patients[b] V: 301 F: 188	N/A	(1) $13,202/recurrence avoided (2) $18,190/recurrence avoided	Sensitive to the recurrence rate, the duration of fidaxomicin treatment and proportion of NAP1/B1/027 strain cases.
Markovic et al, 2014	Cost per patient[b]: V: RSD25,873 F: RSD48,106	Mortality rate[b] V: 0.057 F: 0.050 Total colectomy[b] V: 0.016 F: 0.014	N/A	RSD2,977,621/life-year saved RSD10,276,757/avoided colectomy	F if the outcome is live-year saved, but not for number of avoided colectomies.

Table 5.2 (continued) Results of economic evaluation studies included in the systematic review

Study	Costs	Additional information	QALY/DALY	ICER	Cost-effective intervention
Varier et al, 2015	Cost per patient[b]: V: $3,788 FMT: $1,669	N/A	QALYS by group[b]: V: 0.235 FMT: 0.242	-$302,714/QALY	FMT

QALY: quality-adjusted life year, DALY: disability-adjusted life year, ICER: incremental cost-effectiveness ratio, M: Metronidazole, V: Vancomycin, F: Fidaxomicin, FMT: faecal microbiota transplant, Pl: placebo, Pr: probiotic, N/A: not applicable.
[a] Data inputs [b] Base case results

5.3.4.1 Probiotics

Probiotics are live microorganism that can provide health benefit on the host when administered in adequate doses (Sanders, 2008). *Lactobacillus*, *Bifidobacterium* and *Saccharomyces* are the most common species used for the treatment of CDI (Khanna and Pardi, 2012). A UK trial-based economic evaluation (Allen et al., 2013) concluded that lactobacilli and bifidobacteria given to elderly patients who have been admitted to hospital and exposed to antibiotics, was not cost-effective compared with the use of placebo with an incremental cost-effectiveness ratio (ICER) of £189,662 per QALY gained. Costs were similar in both arms of the trial, but the use of probiotics was not effective in preventing diarrhoea (relative risk 1.04, 95% CI: 0.84-1.28).

5.3.4.2 Screening hospital admissions

A strategy of screening patients at hospital admission by conducting peri-rectal swabbing, pre-amplification in a selective medium and the use of real time-PCR assay for toxin detection, was evaluated in one study (Bartsch et al., 2012a). Patients with positive tests were treated with precautions, including the use of gloves and gowns. Patients who developed CDI received standard therapy (MTZ or VAN). Screening on admission was economically dominant compared to no screening from both hospital and third-party payer perspectives. Different scenarios considering *C. difficile* colonization on admission, contact isolation compliance and probability of infection after colonization rates, all resulted in ICER $\leq$\$256 per QALY gained. The authors adopted a conservative model underestimating the health impact of CDI and

the potential benefits of screening, they also limited the number of CDI episodes, did not include comorbidities and rare complications, and included only costs during hospitalisation. Extrapolating across the US, they suggested that screening for *C. difficile* on admission could save between $152 million to $1.6 billion annually.

5.3.4.3 Vaccines

Lee et al (Lee et al., 2010) constructed a simulation model to analyse the cost-effectiveness of universal vaccination of: (1) all at-risk patients, and (2) only CDI patients receiving antibiotic treatment. Vaccination compared to no vaccination could be dominant for combinations of *C. difficile* risk of colonization >10% based on local prevalence, cost of vaccination between $25 and $100, and vaccine efficacy rates ranged between 25% and 100%. Vaccination was projected to be cost-effective in preventing recurrences if the cost of vaccination was below $800 and efficacy above 50%. The authors concluded that vaccination could prevent both cases and recurrences and save money of society, third-party payers and hospitals, hence supporting investment on this area. However, as a simulation model without evidence on the effectiveness of vaccination, there remains uncertainty as to whether these benefits would be realised in practice.

5.3.4.4 Treatment with fidaxomicin

FDX is indicated for the treatment of CDI having demonstrated lower rates of recurrence and deaths compared to MTZ or VAN. It has been appraised by different agencies, including the NICE (NICE, 2012), AWMSG (AWMSG, 2012) and SMC (SMC, 2012) and is the first line treatment option in some UK hospitals.

Economic evaluations of FDX have considered its comparison with VAN in: CDI patients (Stranges et al., 2013), patients with colitis induced by *C. difficile* who did not respond to MTZ (Marković, 2014), patients with severe CDI or with first recurrence (Nathwani et al., 2014), and patients with severe CDI (Wagner et al., 2014). Bartsch et al (Bartsch et al., 2013) compared the treatment of CDI patients in three scenarios: (1) no FDX, (2) only FDX, and (3) FDX based on strain type. The other study that assessed FDX is presented with FMT findings.

Markovic et al study (Marković, 2014) found FDX to be cost-effective at a cost of RSD2.98 million per life-year gained (threshold of RSD53.3 million per life-year gained) compared with VAN, but not cost-effective when the outcome was the number of avoided colectomies, even if the price is decreased by 50%. This study did not consider patients with severe complications and it was conducted in a low income country where the costs of labour are low compared to the high costs of FDX. The analysis by Nathwani et al (Nathwani et al., 2014) indicated FDX to be cost-effective for both severe CDI (with an ICER of £16,529 per QALY gained) and first CDI recurrence (dominant) compared with VAN. Stranges et al (Stranges et al., 2013) found similar results with an ICER

of $67,576 per QALY gained. However, FDX was no longer cost-effective if cure rates decrease or with improvement in response to oral VAN (Stranges et al., 2013). The model may have overestimated the cost of FDX and recurrence rates in outpatients and underestimated in inpatients. It also did not include the risk of colonization and infection. Wagner et al study (Wagner et al., 2014) concluded that FDX may be a cost-effective option for the treatment of CDI but this was sensitive to clinical cure, recurrence rates and number of cases caused by the NAP1/B1/027 strain. This study took into account only first or second recurrence and severity and length of them were similar to initial episode. In contrast to these studies, Bartsch study (Bartsch et al., 2013) found that FDX was not cost-effective as first line treatment for CDI patient (ICER >$8.8 million per QALY gained). To be cost-effective, FDX needed to cost less than US$150 to treat all CDI patients and between US$160 and US$400 to treat non-NAP/B1/027 cases.

5.3.4.5 Treatment with Faecal microbiota transplant

FMT also known as faecal bacteriotherapy consists of the infusion of a faecal suspension from a healthy donor to restore the balance of bacteria in the gut of patients with recurrent CDI (RCDI). Currently, FMT is indicated for recurrent cases after their third episode of mild or moderate CDI, after the second episode of severe CDI, moderate or severe cases not responding to VAN for 2 weeks or 48 hours, respectively (Bakken et al., 2011), or recurrent cases that have failed on antibiotic therapy or other treatments (NICE, 2014).

Three economic evaluations of FMT were identified. Konijeti et al (Konijeti et al., 2014) assessed the use of FMT in different scenarios for RCDI: (1) FMT via colonoscopy compared with MTZ, VAN and FDX, (2) FMT via duodenal infusion compared with all 3 antibiotic therapies, (3) FMT via enema compared with all 3 antibiotic therapies, (4) FMT all delivery via compared with all 3 antibiotic therapies, and (5) the 3 antibiotic therapies alone. Varier et al compared the use of FMT with MTZ and VAN for initial CDI (Varier et al., 2014), and compared FMT to VAN at the third RCDI (Varier et al., 2015).

FMT via colonoscopy was the most cost-effective option among those assessed by Konjieti et al (Konijeti et al., 2014), with an ICER of $17,016 per QALY gained compared with antibiotic therapy. Varier et al (Varier et al., 2014) concluded that FMT via colonoscopy is cost-effective (dominant) compared with VAN, but not cost-effective compared with MTZ (ICER $124,964 per QALY gained) in initial CDI. Moreover, in third RCDI, FMT is less costly and more effective (dominant) compared with VAN (Varier et al., 2015). While the first study (Konijeti et al., 2014) compared all antibiotic therapies and different methods of FMT delivery, Varier et al (Varier et al., 2014, Varier et al., 2015) did not include FDX. Moreover, they considered only patients without serious conditions and FMT delivered only via colonoscopy.

5.4 Discussion

This review identified 11 economic evaluation studies on CDI prevention with the use of probiotics, vaccination of patients, and screening hospital admission, and on treatment with FDX and FMT. Interventions were

considered cost-effective depending on different scenarios, e.g. perspectives, costs, intervention to be compared. The use of probiotics was the only intervention not cost-effective. Only treatment with FDX and FMT were assessed in more than one study; FMT was compared with standard therapy in three studies conducted by two different groups whose findings were similar, FDX was assessed by five different groups and although only one study found it not cost-effective, in general their findings were variable.

Probiotics were not considered effective or cost-effective in the trial, however stool samples were not tested for *C. difficile* toxin in 41% of patients which may account for the low rate of CDI (~1%). Consequently, NICE, Food and Drug Administration (FDA), SHEA and IDSA do not recommend its use for preventing recurrent CDI (Cohen et al., 2010, Surawicz et al., 2013, PHE, 2013). However, further research is needed to confirm the findings, identify the population at risk of CDI and determine the influence of probiotics on the quality of life (38).

One study found that a strategy of screening on admission followed by antibiotic treatment to be a cost-effective approach even with high probabilities of colonization on admission or infection after colonization, and lower contact isolation compliance rate. Although routine screening in hospitalised patients without diarrhoea is not recommended (Bartsch et al., 2012a, Bartsch et al., 2012b) detection of asymptomatic carriers may reduce the transmission and the number of new colonisations and CDI cases (Lanzas and Dubberke, 2014). This study also suggested additional benefit of screening as more attention is given to the cleaning of previously occupied rooms. Decision makers should consider this strategy when colonization is present in ≥10% of

patients with identifiable risk factors (Leekha et al., 2013), however, further economic evidence is warranted to assess the value of routine surveillance for controlling transmission and preventing new cases.

Vaccines from Pfizer and Valneva are currently in development. There is still no published information about prices, efficacies and target population but the decision analytic model considered a wide range of plausible values and indicated vaccination as a potentially cost-effective treatment for prevention of cases and recurrences, showing promising results for recurrences even with high prices.

Included economic evaluations of FDX were conducted in US, Canada, UK and Serbia and varied in terms of healthcare system and costs, patient characteristics, rates of transmission and endemicity (Marković, 2014). Cost perspectives and time horizon of analysis were different across studies making comparisons among studies difficult. However, with the exception of one study (Bartsch et al., 2013), FDX was generally cost-effective. Two studies (Bartsch et al., 2013, Wagner et al., 2014) concluded that FDX was not the most cost-effective treatment option for first episode and first recurrence of patients infected by NAP1/B1/027 strains mainly owing to the high price of FDX (Bartsch et al., 2013). This situation might change with the future availability of generic FDX. While FDX is recommended as first line treatment in some UK hospitals, there remains uncertainty surrounding its cost-effectiveness in this context.

FMT via colonoscopy is cost-effective compared with standard antibiotic therapy in various contexts of use, i.e. first recurrence, initial treatment and,

third recurrence, following the American guideline (Surawicz et al., 2013). All studies were conducted in the US. As with any decision analytic models, several assumptions were required and although there are differences between models, their findings were similar and could indicate a new treatment option for initial CDI and RCDI. However, the potential for the use of FMT requires further confirmatory evidence as not all costs related to the FMT process were included in the economic evaluations and it is possible that other methods of delivery could be more efficient and safer. NICE also recommends more research related to optimal dosage, mode of administration and choice of donor (NICE, 2014). A clinical trial completed recently is evaluating the potential cost-effectiveness of faecal microbial transplant in the first episode of CDI (NIH, 2017).

Our review has limitations. Firstly, the evidence base is not extensive, being limited to 11 studies. This may indicate that interventions for prevention other than FDX are not effective. While the number of CDI cases has been decreasing in recent years, effective measures for infection control remains challenging in some healthcare settings and evidence on clinical effectiveness alone are often not sufficient to support the introduction of health technologies (Rawlins and Culyer, 2004).

Secondly, as with any review of economic evaluations conducted in different jurisdictions, meaningful comparison between studies are not possible because of differences in practice, healthcare systems, patient case-mix and disease characteristics.

Finally, methodological weaknesses in the studies were mainly related to the models employed, which represent the decision problem, but are typically reliant on a number of assumptions and are limited in the range of outcomes, costs and interventions that may be compared. Comorbidities were not usually included in the models. No data of FMT efficacy for initial CDI were available and RCDI data were used instead. There are no health utilities published for CDI, thus in all modelling studies those values were estimated comparing with other causes of diarrhoea. There were limitations in respect to the reporting of analyses, with items such as time horizon, population and setting and location not reported in some studies.

The review identified studies which suggest the following interventions may be cost-effective: screening patients on hospital admission, vaccination (but depending on cost, efficacy and *C. difficile* risk), FMT via colonoscopy, and FDX in some specific conditions, as price and duration of treatment. The use of probiotics to prevent CDI was the only intervention measure considered not cost-effective in this review. The lack of studies identified in this review and the focus on treatment in the majority reinforces the need and importance of economic studies in the prevention of CDI.

Chapter 6

Cost of illness analysis of *Clostridium difficile* infection in Liverpool in 2008-2012 and 2012-2016

6.1 Introduction

As mentioned before, the 2006-2008 outbreak caused by the spread of hypervirulent strain RT027 was associated with severe illness and increased death and recurrence rates. The transition between an epidemic to an endemic phase was observed after 2008 when the number of cases dropped from 33,000 in 2007/2008 to 20,000 in 2008/2009, reaching a stable incidence after 2013/2014 when 5,000 cases where reported in England (PHE, 2017).

Comprehensive reports of costs associated with *C. difficile* related hospitalisation in the UK are limited to two studies (Wilcox et al., 2017, Wilcox et al., 1996). The original 1996 analysis covers the period prior to the emergence of major outbreaks in North America and Europe. This study included 50 CDI cases recruited between 1994 and 1995 and additional costs for these patients compared to matched controls were £4,107. Costs were based on average difference in LoS, use of antibiotics and laboratory tests between groups. The same group recently published another study including 64 CDI recurrent patients and 64 first CDI case patients between 2012 and 2014 and median costs per patient during 28 day post-index period were £7,539 (£5,617-9,730) and £6,294 (£2,700-9,216). Hospital bed night was the major cost component (87%) in this study, as costs varied between £275 (medical ward) and £1,400 (intensive care) per night. Additionally, investigations, procedures and laboratory costs were considered in the cost calculation.

The economic impact of *Clostridium difficile* on the NHS of several epidemic seasons has yet to be determined in more detail as no studies have included

the period prior or post the RT027 outbreak. Thus, the aim of this chapter was to estimate costs associated with CDI episodes through different seasons (2008-2012 and 2012-2016) in hospitalised patients at RLBUHT from the perspective of the healthcare provider/hospital.

6.2 Patients and methodology

6.2.1 Patient cohort

Cohort and audit patients from phases I and II were included in this study. The methods of patient recruitment are described in section 2.2.1.

6.2.2 Data collection

Demographic (age, gender and IMD score), clinical (CCI score, mode of acquisition, CDI severity and CDI recurrence) and hospitalisation data (hospitalisation periods, mortality rates and hospitalisation costs) described in section 2.2.3, were used in this chapter.

For phase II patients, the health status was measured by the EuroQol five dimension questionnaire (EQ-5D-3L), and the visual analogue scale (EQ-VAS). The EQ-5D-3L asks patients about their perceptions related to mobility, self-care, usual activities, pain and discomfort and anxiety and depression according to three levels (no, some, extreme problems) (van Reenen and Janssen, 2015). Combined results were used to obtain single index value with 5-digit number and were converted to a value set between 0 (death) and 1 (full health) according to the UK population (Szende and Williams, 2004, van

Reenen and Janssen, 2015). The EQ-VAS uses a scale from 0 to 100 to indicate how the health is on the day (van Reenen and Janssen, 2015).

6.3 Statistical analysis

6.3.1 Patient characteristics

Descriptive analyses were undertaken to assess the difference in demographic, clinical and hospitalisation characteristics between CDI cases (TOX+) and control patients (TOX-) for phase I, and between CDI cases (GDH+/TOX+), potential *C. difficile* carriers (GDH+/TOX-) and diarrhoea controls (GDH-/TOX-) for phase II. Data were described by mean and 95% confidence intervals (CI) for continuous variables and frequency (F) and percentage (P) for categorical variables. Hospitalisation costs calculation were described in the section 2.2.4 and were presented as the nearest pound sterling (£) and for hospitalisation costs and hospitalisation period, a non-parametric bootstrap sampling with 2,000 replications was used to estimate CIs and bias corrected and accelerated (Bca) confidence intervals were presented. T-test or ANOVA test were employed to compare normally distributed continuous variables, Mann-Whitney U test or Kruskal Wallis test to compare non-normally distributed continuous variables, and chi-square test to compare categorical variables. Different tests were employed for different phases as phase I has 2 groups and phase II has 3 groups.

6.3.2 Multivariable analysis

Outliers (<2.5 and >97.5 percentiles) of outcome variables were excluded from the model after confirmation that those patients were exceptions compared to the whole cohort and with unrelated diagnostic and high cost medical conditions and treatment, e.g. leukaemia, myeloma, lymphoma, rheumatoid arthritis. Low costs were related to day case patients. As costs data are skewed and mean costs are the interest, a generalized linear model (GLM) was the method chosen to evaluate the cost difference associated with the infection by *C. difficile*. Studies have shown that GLM is a good option to assess costs and deal with non-normally distributed outcomes instead of employing normal and bootstrapped multiple linear regression, median regression or normal linear regression of log costs (Dodd et al., 2006, Moran et al., 2007, Barber and Thompson, 2004). Potential covariates were used to control the analysis and varied according to the different models. Covariates were chosen according to relevance for the outcome and based on individual significance after univariate analysis. The final model was built using stepwise regression with use of $p \leq 0.05$ for inclusion criteria and $p > 0.05$ for exclusion criteria.

Gamma distribution and link log is usually the model chosen to assess costs. However, modified Park test was used to assess appropriateness of family chosen for each model, with a non-significant p-value and a small value of chi-squared indicating the family to be chosen (Manning and Mullahy, 2001, Jones, 2010). Model performance was assessed through graphical analysis using a probability of standard deviance residual distribution against costs and a plot of square root of standard deviance residuals against predicted values

to confirm the normality and homoscedasticity of model residuals, respectively (Montgomery et al., 2012, Jones, 2010). The first plot should present an approximate straight line and the second plot should show an equal variability across the variables.

Cohort patients were tested alone in a first model and with audit patients in a second model, also the different phases (July 2008 – March 2012 and April 2012 – January 2016) were analysed separately as shown in Table 6.1. These phases represent different epidemiological periods and may incur different hospitalisation costs. Moreover, a new diagnostic test (GDH) and the use of FDX as a first-line treatment were implemented in 2012 when recruitment of phase II patients started. Also, models with both cohort and audit patients (3 and 4) included only those who had hospitalisation information and costs available obtained from information and finance departments. Thus, patients with missing data were not considered in the models. All data were analysed using STATA version 14.0 (StataCorp LP, College Station, Texas). Statistical significance was set at $p<0.05$.

Table 6.1 Patients included for models performed in this chapters

Model	Phase	Year	Recruitment	Patients
1	I (epidemic)	2008-2012	cohort	257 CDI cases 139 controls
2	II (endemic)	2013-2015	cohort	78 CDI cases 57 potential carriers 164 controls
3	I (epidemic)	2008-2012	cohort audit	633 CDI cases 132 controls
4	II (endemic)	2012-2016	cohort audit	204 CDI cases 383 potential carriers 3,703 controls

6.4 Results

6.4.1 Cohort phase I recruitment

6.4.1.1 Patient characteristics

257 CDI cases and 139 antibiotic-associated controls were recruited in phase I. Their demographic, clinical and hospitalisation characteristics and laboratory results are presented in Table 6.2. CDI case patients were significantly older than controls [70 (68-72) vs 65 (62-68) years, p=0.011], but no difference in gender was shown between groups and most of the patients were female. Disease was severe in 42% (n=107) of cases and CDI recurrence presented in 18% (n=47) cases.

Mean LoS was 39.9 days (95% CI: 34.9-47.2) for cases patients and 23.6 days for controls (95% CI: 19.8-29.7, p<0.001) and disease period was higher for CDI cases [26.3 (95% CI: 21.5-31.1) vs 14.7 days (95% CI: 11.7-20.0), p<0.001]. Time of hospitalisation within 6 months prior to index episode for CDI cases was twice as high as for control patients [12.4 (95% CI: 9.6-15.2) vs 6.4 (95% CI: 4.5-9.4) days, p=0.001]. Case group presented higher mortality rates; there was no significant difference in mortality within 4 weeks between case and control group [7% (n=18) vs 4% (n=5), p=0.167), but the difference was significant during hospitalisation [9% (n=24) vs 4% (n=5), p=0.036] and within 1 year [31.5% (n=81) vs 17% (n=24), p=0.002]. Mean unadjusted hospitalisation costs were significantly higher for cases [£6,247 (95% CI: 5,649-6,942) vs £4,141 (95% CI: 3,689-4,779), p=0.003] when omitting outliers (costs model). Hospitalisation costs during 6 months prior to index episode (LoS before) were also higher for CDI group than diarrhoea

Table 6.2 Patient characteristics of cohort phase I

	control (n=139)		CDI case (n=257)		
	n	M (95% CI) F (P)	n	M (95% CI) F (P)	p
Demographics					
Age (years)	139	65 (62-68)	257	70 (68-72)	0.011
gender (female, %)	139	78 (56.1)	257	149 (58.0)	0.721
IMD (score)	135	39.8 (36.2-43.4)	229	38.0 (35.4-40.9)	0.438
Clinical					
CCI (score)	139	1.2 (1.1-1.5)	256	1.4 (1.2-1.7)	0.270
mode of acquisition (HCAI, %)	139	102 (73.4)	257	161 (62.6)	<0.001
CDI severity (%)	0		257	107 (41.6)	.
CDI recurrence (%)	0		257	47 (18.3)	.
Hospitalisation					
LoS (days)[a]	139	23.6 (19.8-29.7)	257	39.9 (34.9-47.2)	<0.001
disease (days)[a]	139	4.75 (11.7-20.0)	257	26.3 (22.5-32.5)	<0.001
pre-test (days)[a]	139	8.9 (6.9-12.9)	257	13.6 (11.4-16.8)	0.160
LoS before (days)[a]	139	6.4 (4.5-9.4)	257	12.4 (9.8-15.5)	0.001
LoS after (days)[a]	139	12.9 (8.1-30.0)	257	10.2 (7.4-13.5)	0.508
Time to death (days)	24	144 (107-188)	81	111 (92-134)	0.182
Mortality hospitalisation (%)	139	5 (3.6)	257	24 (9.3)	0.036
4 weeks (%)	139	5 (3.6)	257	18 (7.0)	0.167
1 year (%)	139	24 (17.3)	257	81 (31.5)	0.002
Costs index (£)[a]	132	£4,726 (4,082-5,655)	217	£7,721 (6,770-9,343)	<0.001
model (£)[a]	126	£4,141 (3,689-4,779)	203	£6,246 (5,649-6,942)	0.003
before (£)[a]	132	£2,532 (1,836-3,632)	204	£4,153 (3,164-5,869)	0.047
after (£)[a]	132	£3,487 (2,264-6,484)	204	£2,680 (2,096-3,377)	0.820

CDI: *Clostridium difficile* infection, IMD: index of multiple deprivation score, CCI: Charlson comorbidity index, HCAI: healthcare-associated infection, LoS: length of stay, LoS before: number of hospitalised days 6 months prior to index hospitalisation, LoS after: number of hospitalised days 6 months post to index hospitalisation, Costs index: costs of index hospitalisation, Costs model: hospitalisation costs excluding outliers (5%), Costs before: costs of hospitalisation 6 months prior to index hospitalisation, Costs after: costs of hospitalisation 6 months post to index hospitalisation.
[a] bias corrected and accelerated confidence intervals

6.4.1.1 Multivariable analysis

A univariate analysis (Table 6.3) was performed to help choose the covariates. Age, gender and mortality during hospitalisation were included in all models as they were considered potential confounders. Initially, CDI status (case or control), age, gender, CCI, mode of acquisition, disease and pre-test periods and LoS before, mortality during hospitalisation were included in the model as covariates.

Table 6.3 Univariate analysis of cohort phase I patients

	n	β (SE)	exp^{β} (95% CI)	p
CDI status	349	0.49 (0.12)	1.63 (1.29-2.07)	<0.001
age	360	-0.00 (0.00)	0.99 (0.99-1.00)	0.068
gender	358	-0.06 (0.13)	0.94 (0.73-1.21)	0.611
IMD	345	0.00 (0.00)	1.00 (1.00-1.01)	0.904
CCI	359	-0.05 (0.04)	0.95 (0.88-1.03)	0.219
mode of acquisition	360	0.77 (0.12)	2.16 (1.70-2.75)	<0.001
disease	360	0.02 (0.00)	1.02 (1.01-1.02)	<0.001
pre-test	360	0.02 (0.00)	1.02 (1.02-1.03)	<0.001
LoS before	360	0.00 (0.00)	1.00 (1.00-1.01)	0.713
Mortality hospitalisation	360	0.32 (0.24)	1.38 (0.87-2.20)	0.193
4 weeks	360	-0.45 (0.25)	0.64 (0.39-1.04)	0.071
1 year	360	-0.05 (0.14)	0.95 (0.73-1.24)	0.707

IMD: index of multiple deprivation score, CCI: Charlson comorbidity index, LoS before: number of hospitalised days within 6 months prior to index hospitalisation.

The stepwise process to choose the best model is summarised in Table 6.4. The variables excluded from the model were mortality during hospitalisation, CCI, age, gender and LoS before.

Table 6.4 Stepwise process to choose the best model of phase I

Initial	CDI status, age, gender, CCI, mode of acquisition, disease, pre-test, LoS before, mortality hospitalisation				
Final	CDI status, mode of acquisition, disease, pre-test, LoS before				
	Step	Residual df	AIC	BIC	p
1	Initial	323	18.96	-1808.58	
2	mortality hospitalisation	324	18.96	-1814.39	0.915
3	CCI	325	18.95	-1820.19	0.865
4	age	326	18.95	-1825.66	0.247
5	gender	327	18.94	-1831.25	0.344
6	LoS before	328	18.94	-1836.31	0.080

CCI: Charlson comorbidity index, Before LoS: number of hospitalised days within 6 months prior to index hospitalisation.

Gamma distribution whose variance is proportional to the square of the mean was chosen as family and performed with a link log. The relationship between outcome and predictors is defined as: $E(y)=\exp(\beta_0+\beta_1X_1+\beta_2X_2+\ldots)$. In this model, presence of toxin (expβ=1.17, 95% CI: 1.05-1.31), increased disease period (expβ=1.02, 95% CI: 1.02-1.02) and pre-test period (expβ=1.02, 95% CI: 1.01-1.02) and health-care associated infections (expβ=1.30, 95% CI: 1.13-1.49) were associated with higher hospitalisation costs of cohort phase I patients (Table 6.5). Between 2008 and 2012, control patients with diarrhoea by no confirmed CDI cost £4,924±3,568 while a CDI case patient incurred higher cost of £5,761±4,176.

Table 6.5 GLM with family gamma and link log model of cohort phase I

	β (SE)	**expβ (95% CI)**	**p**
CDI status	0.16 (0.06)	1.17 (1.05-1.31)	0.006
mode of acquisition	0.26 (0.07)	1.30 (1.13-1.49)	<0.001
Disease	0.02 (0.00)	1.02 (1.02-1.02)	<0.001
pre-test	0.02 (0.00)	1.02 (1.01-1.02)	<0.001

Before LoS: number of hospitalised days within 6 months prior to index hospitalisation.

To assess the goodness of fit of the model, the modified Park test was performed (Table 6.6) and showed gamma as the best choice for the model. Plot of probability of standard deviance residual distribution against costs and a plot of square root of standard deviance residuals against predicted values (Figure 6.1 and 6.2) were analysed and both normality and homoscedasticity of model residuals were confirmed.

Table 6.6 Modified Park test to assess choice of family for GLM model of cohort phase I

	Chi2	P
Gaussian	42.16	<0.001
Poisson	5.98	0.014
Gamma	2.56	0.109
Inverse Gaussian	31.89	<0.001

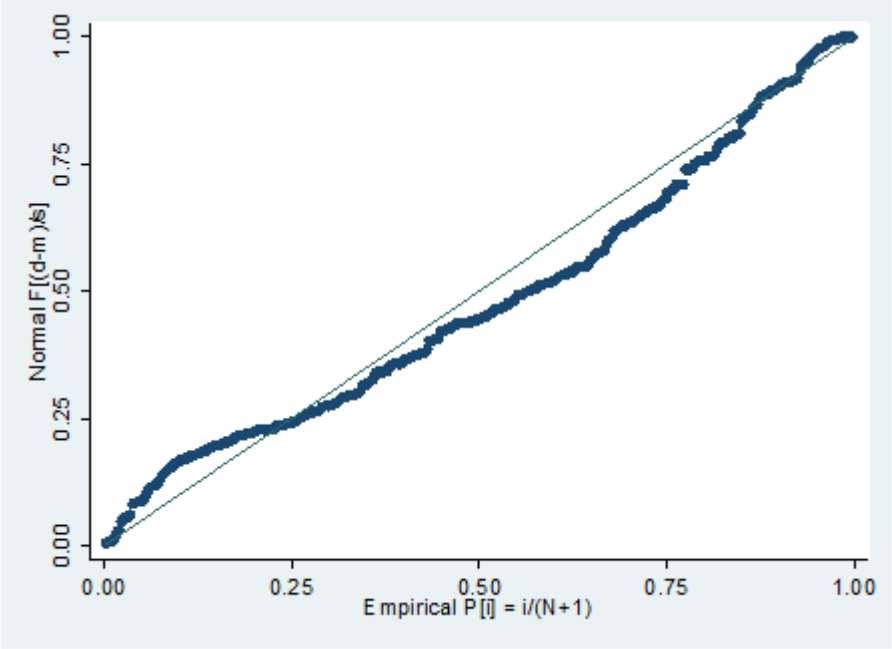

Figure 6.1 Plot of probability of standard deviance residual distribution against costs of cohort phase I model

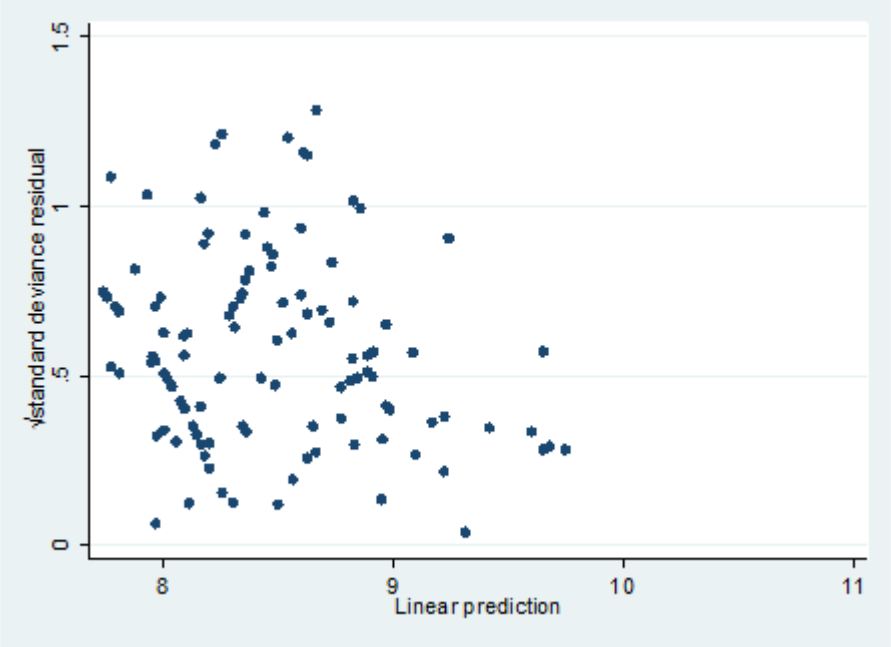

Figure 6.2 Plot of square root of standard deviance residual against linear prediction of cohort phase I model

6.4.2 Phase II cohort

6.4.2.1 Patient characteristics

78 CDI cases, 57 *C. difficile* carriers and 164 controls were recruited in phase II cohort and all patients' characteristics are summarized in Table 6.7. There was no difference on age, gender and IMD score between groups, but CCI was higher for cases than carriers and controls [1.9 (95% CI: 1.5-2.3) vs 1.3 (95% CI: 0.9-1.6) vs 0.5 (95% CI: 0.3-0.6), p<0.001). Mean LoS, disease and pre-test periods were similar on cases and carriers and significantly higher than controls [LoS 31.8 (95% CI: 23.6-48.8) vs 30.9 (95% CI: 23.3-41.7) vs 8.8 95% CI: 7.2-10.9) days, p<0.001; disease period 18.4 95% CI: 14.1-24.8) vs 19.0 (95% CI: 14.4-26.2) vs 8.0 days (95% CI: 6.8-9.8), p<0.001; pre-test 13.4 (95% CI: 8.8-23.6) vs 11.9 (95% CI: 7.3-17.7) vs 3.2 days (95% CI: 2.3-5.2), p=0.003]. Hospitalisation prior to index hospitalisation was higher on cases

than other groups [10.8 (95% CI: 7.5-16.6) vs 8.2 (95% CI: 4.4-16.0) vs 2.4 95% CI: 1.2-4.5), p<0.001]. Although there was no difference in mortality within 4 week, case patients were more likely to die during hospitalisation [8% (n=6) vs 4% (n=2) vs 1% (n=2), p=0.032] and within 1 year [41% (n=32) vs 28% (n=16) vs 4% (n=6), p<0.001) compared to potential carriers and control patients.

Excluding outliers, mean unadjusted costs of case group were £6,056 (95% CI: 5,228-7,248), potential carrier group £5,745 (95% CI: 4,491-7,713) and control group £3,374 (95% CI: 3,000-3,902, p<0.001). Cost of hospitalised days within 6 months prior to index hospitalisation were higher for potential carriers [£6,363 (95% CI: 3,616-12,868)] compared to cases [£4,922 (95% CI: 3,251-7,833)] and controls [£1,679 (95% CI: 849-3,806), p<0.001].

The two instruments to measure health status showed a better health state according to patient's view in control patients than carriers and cases [EQ-5D-3L 0.661 (95% CI: 0.592-0.729) vs 0.590 (95% CI: 0.499-0.681) vs 0.441 (95% CI: 0.334-0.549), p>0.001; EQ-VAS adapted 57.4 (95% CI: 52.1-62.6) vs 56.1 (95% CI: 50.3-61.9) vs 46.0 (95% CI: 40.4-51.5), p<0.001]. Compared to national population norm aged between 55 and 64 years, all groups had lower values of EQ-5D-3L (0.804-0.819) and EQ-VAS (81.7). Moreover, results of EQ-5D-3L by CDI status are shown on Table 6.8 and CDI cases showed higher rates of extreme problems in all dimensions. Usual activities and self-care were dimensions that CDI cases had more problems while diarrhoea control and potential carrier patients had more problems in usual activities and pain dimensions.

Table 6.7 Patients characteristics of cohort phase II

	control (n=164)		carrier (n=57)		case (n=78)		
	n	M (95% CI) / F (P)	n	M (95% CI) / F (P)	n	M (95% CI) / F (P)	p
Demographics							
Age (years)	164	62 (59-64)	57	59 (54-63)	78	65 (61-68)	0.101
gender (female, %)	164	85 (51.8)	57	30 (52.6)	78	35 (44.9)	0.550
IMD (score)	146	34.8 (31.2-38.3)	50	33.9 (28.1-40.9)	75	33.2 (29.1-37.9)	0.870
Clinical							
CCI (score)	164	0.5 (0.3-0.6)	57	1.3 (0.9-1.6)	78	1.9 (1.5-2.3)	<0.001
mode of acquisition (HCAI, %)	164	106 (64.6)	57	33 (57.9)	78	39 (50.0)	0.032
CDI severity (%)	0		0		78	34 (43.6)	.
CDI recurrence (%)	0		0		78	7 (9.0)	.
Hospitalisation							
LoS (days)[a]	164	8.8 (7.2-10.9)	57	30.9 (23.3-41.7)	78	31.8 (23.6-48.8)	<0.001
disease (days)[a]	164	8.0 (6.8-9.8)	57	19.0 (14.4-26.2)	78	18.4 (14.1-24.8)	<0.001
pre-test (days)[a]	164	3.2 (2.3-5.2)	57	11.9 (7.3-17.7)	78	13.4 (8.8-23.6)	0.003
LoS before (days)[a]	164	2.4 (1.2-4.5)	57	8.2 (4.4-16.0)	78	10.8 (7.5-16.6)	<0.001
LoS after (days)[a]	164	12.6 (6.0-25.6)	57	10.1 (4.2-27.0)	78	8.7 (4.3-22.6)	0.834
Time to death (days)	6	122 (55-196)	16	149 (111-202)	32	132 (100-165)	0.777
Mortality hospitalisation (%)	164	2 (1.2)	57	2 (3.5)	78	6 (7.7)	0.032
4 weeks (%)	164	2 (1.2)	57	1 (1.8)	78	3 (3.8)	0.391
1 year (%)	164	6 (3.7)	57	16 (28.1)	78	32 (41.0)	<0.001
Costs index (£)[a]	164	£3,374 (3,000-3,902)	57	£12,845 (8,922-18,274)	78	£7,778 (6,361-10,8827)	<0.001
model (£)[a]	84	£3,374 (3,000-3,902)	57	£5,745 (4,491-7,713)	64	£6,056 (5,228-7,248)	<0.001
before (£)[a]	164	£1,679 (849-3,806)	57	£6,363 (3,616-12,868)	78	£4,922 (3,251-7,833)	<0.001
after (£)[a]	164	£3,881 (2,351-6,989)	57	£4,683 (2,022-11,101)	78	£2,9998 (1,722-6,152)	0.640
Economics							
EQ-5D	85	0.661 (0.592-0.729)	48	0.590 (0.499-0.681)	66	0.441 (0.334-0.549)	<0.001
EQ-VAS	85	57.4 (52.1-62.6)	48	56.1 (50.3-61.9)	66	46.0 (40.4-51.5)	<0.001
Medicines							
Fidaxomicin (%)	164	0	57	26 (45.6)	78	51 (65.4)	<0.001

Table 6.8 Scores of EQ-5D-3L dimensions by CDI status

Dimension	Problems	Diarrhoea control	Potential carriers	CDI cases
Mobility	No	55 (49.1)	24 (48.0)	24 (32.4)
	Some	56 (50.0)	25 (50.0)	37 (50.0)
	Extreme	1 (0.9)	1 (2.0)	13 (17.6)
Self-care	No	92 (82.1)	40 (80.0)	39 (52.7)
	Some	18 (16.1)	8 (16.0)	15 (20.3)
	Extreme	2 (1.8)	2 (4.0)	20 (27.0)
Usual activities	No	59 (52.7)	21 (42.0)	17 (23.0)
	Some	44 (39.3)	19 (38.0)	28 (37.8)
	Extreme	9 (8.0)	10 (20.0)	29 (39.2)
Pain Discomfort	No	36 (31.9)	19 (38.0)	33 (44.6)
	Some	53 (46.9)	24 (48.0)	29 (39.2)
	Extreme	24 (21.2)	7 (14.0)	12 (16.2)
Anxiety Depression	No	70 (62.5)	27 (54.0)	43 (58.1)
	Some	39 (34.8)	22 (44.0)	23 (31.1)
	Extreme	3 (2.7)	1 (2.0)	8 (10.8)

6.4.2.2 Multivariable analysis

After univariate analysis (Table 6.9), the covariates used in initial model were CDI status, age, gender, CCI, IMD score, mode of acquisition, disease and pre-test periods, number of hospitalised days within 6 months prior the index hospitalisation and mortality during hospitalisation.

Table 6.9 Univariate analysis of cohort phase II patients

	n	β (SE)	expβ (95% CI)	p
CDI status	200	0.45 (0.12)	1.57 (1.23-2.01)	<0.001
age	213	-0.00 (0.00)	1.00 (0.99-1.01)	0.618
gender	213	-0.12 (0.19)	0.89 (0.61-1.30)	0.540
IMD score	210	-0.01 (0.00)	0.99 (0.98-1.00)	0.021
CCI	213	0.19 (0.06)	1.21 (1.07-1.37)	0.003
mode of acquisition	203	0.79 (0.17)	2.21 (1.58-3.10)	<0.001
disease	203	0.04 (0.01)	1.04 (1.02-1.05)	<0.001
pre-test	203	0.03 (0.01)	1.03 (1.01-1.05)	<0.001
LoS before	213	0.00 (0.00)	1.00 (0.99-1.02)	0.419
Mortality hospitalisation	213	1.12 (0.47)	3.06 (1.22-7.64)	0.017
4 weeks	213	0.69 (0.57)	2.00 (0.65-6.15)	0.225
1 year	213	0.56 (0.22)	1.74 (1.12-2.71)	0.013
EQ-5D-3L	199	-0.32 (0.26)	0.73 (0.44-1.22)	0.226
EQ-VAS	193	-0.01 (0.00)	0.99 (0.98-1.00)	0.097
Fidaxomicin	213	0.56 (0.21)	1.75 (1.15-2.65)	0.009

IMD: index of multiple deprivation score, CCI: Charlson comorbidity index, LoS before: number of hospitalised days within 6 months prior to index hospitalisation, EQ-5D-3L: EuroQol five dimensions questionnaire**,** EQ-VAS: EuroQol visual analogue scales.

Table 6.10 Stepwise process to choose the best model of cohort phase II

Initial	CDI status, age, gender, CCI, IMD, mode of acquisition, disease, pre-test, LoS before, mortality hospitalisation				
Final	CDI status, disease, pre-test				
	Step	Residual df	AIC	BIC	p
1	Initial	175	18.79	-884.14	
2	IMD	176	18.78	-889.36	0.960
3	mortality hospitalisation	177	18.77	-894.59	0.920
4	CCI	178	18.76	-899.77	0.612
5	gender	179	18.75	-904.90	0.440
6	age	180	18.74	-910.01	0.408
7	LoS before	181	18.73	-914.98	0.194
8	mode of acquisition	182	18.72	-919.82	0.125

CCI: Charlson comorbidity index, LoS before: number of hospitalised days within 6 months prior to index hospitalisation, IMD: index of multiple deprivation score.

Table 6.10 shows the stepwise process with all excluded variables from the model. Thus, the final model included only CDI status and disease and pre-test periods. As patients who were treated with FDX had the costs of antibiotic

treatment added to costs of hospitalisation, the interaction between CDI status and use of FDX was used in the model.

The best model to predict the costs of patients was a GLM with gamma distribution and link log and had included CDI status interacting with the use of FDX and disease and pre-test periods (Table 6.11). In this model, the variables positively associated with outcome were treatment with FDX in a GDH positive test (expβ=1.36, 95% CI: 1.09-1.69), treatment with FDX and presence of toxin positive test (1.51, 95% CI: 1.30-1.75) and increased disease (expβ=1.02, 95% CI: 1.02-1.03) and pre-test (expβ=1.01, 95% CI: 1.01-1.02) periods. During the period of 2013 and 2015, hospitalisation costs were on average £4,227±4,963 for diarrhoea control patients, £4,504±5,288 for potential carrier patients that did not receive FDX, £5,746±6,746 for potential carrier patients who received FDX, £5,011±5,883 for CDI cases not treated with FDX and £6,355±7,473 for CDI case patients treated with FDX.

Table 6.11 GLM with family gamma and link log model of cohort phase II

	β (SE)	**expβ (95% CI)**	**p**
NF - GDH+/TOX-[a]	0.06 (0.10)	1.07 (0.88-1.29)	0.514
F - GDH+/TOX-[a]	0.31 (0.11)	1.36 (1.09-1.69)	0.005
NF - GDH+/TOX+[b]	0.17 (0.10)	1.19 (0.97-1.45)	0.098
F - GDH+/TOX+[b]	0.41 (0.08)	1.51 (1.30-1.75)	<0.001
disease	0.02 (0.00)	1.02 (1.02-1.03)	<0.001
pre-test	0.02 (0.00)	1.02 (1.01-1.02)	<0.001

NF: no fidaxomicin treatment, F: Fidaxomicin treatment
[a] potential carrier patients [b] CDI case patients

Gamma distribution was confirmed to be more appropriate family for the model when assessing modified Park test (Table 6.12). Normality and

homoscedasticity of the residual were also confirmed through graphs of goodness of model fit (Figure 6.3 and 6.4).

Table 6.12 Modified Park test to assess choice of family for GLM model of cohort phase II

	Chi2	p
Gaussian	105.45	<0.001
Poisson	33.34	<0.001
Gamma	1.64	0.201
Inverse Gaussian	10.34	0.001

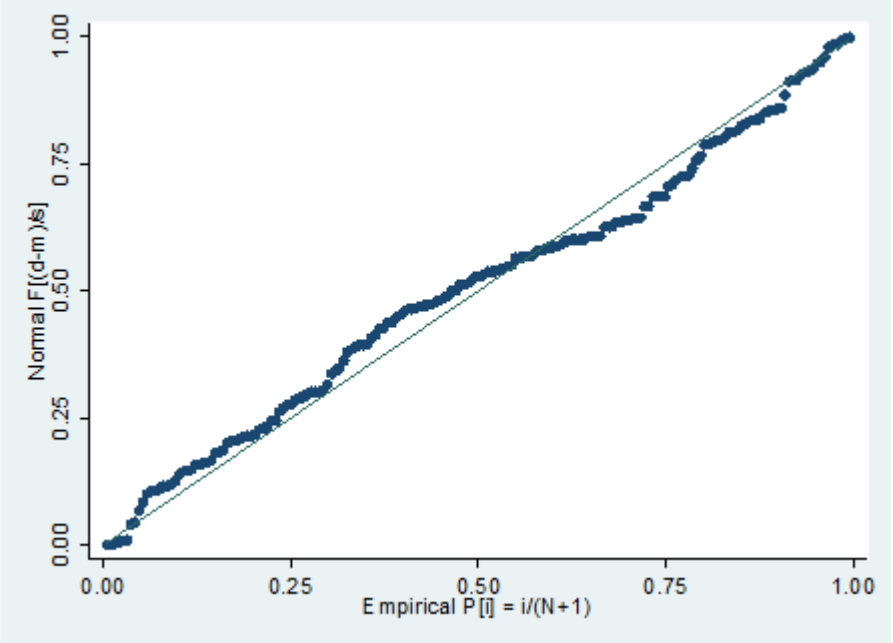

Figure 6.3 Plot of probability of standard deviance residual distribution against costs of cohort phase II model

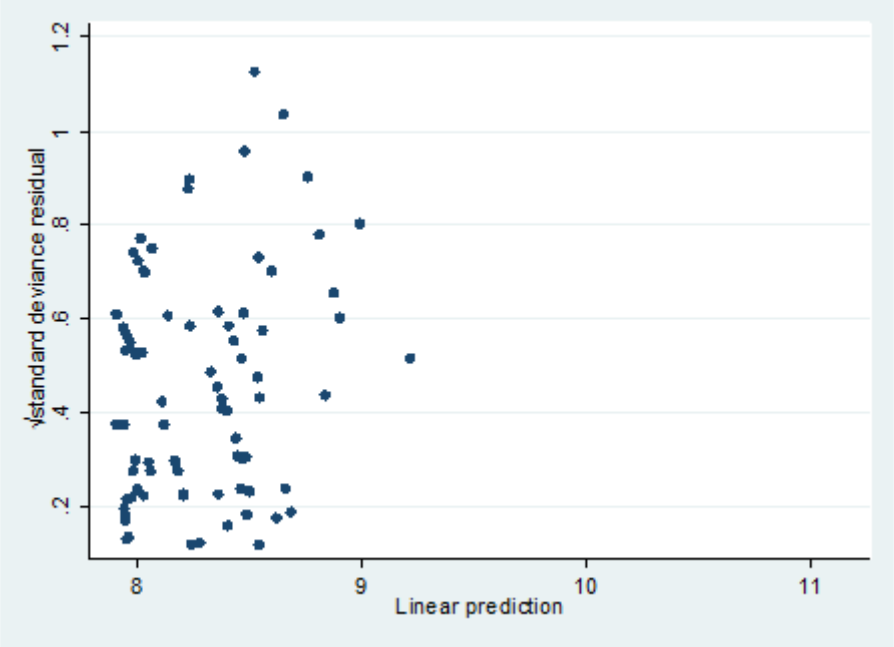

Figure 6.4 Plot of square root of standard deviance residual against linear prediction of cohort phase II model

6.4.3 Phase I cohort and audit patients

6.4.3.1 Patient characteristics

As only cases patients were included in the cohort group, this analysis included 633 case patients and 132 control patients with available hospitalisation costs (Table 6.13). Case patients were older than controls [74 (95% CI: 73-75) vs 65 (95% CI: 62-68) years, p<0.001) and they stayed hospitalised more time during index hospitalisation [LoS 39.1 (95% CI: 36.0-42.8) vs 21.2 days (95% CI: 17.7-26.1), p<0.001; disease period 23.5 (95% CI: 21.5-26.4) vs 12.5 days (95% CI: 10.2-16.8), p<0.001; pre-test period 15.6 (95% CI: 14.0-17.6) vs 8.6 days (95% CI: 6.6-12.4), p<0.001); and within 6 months before it 13.1 (95% CI: 11.5-15.4) vs 6.4 (95% CI: 4.5-9.8) days, p=0.003]. Mortality rates were higher in case patients in all scenarios: during hospitalisation [29% (n=181) vs 4% (n=5), p<0.001]; within 4 weeks [24%

(n=155) vs 5% (n=6), p<0.001]; and within 1 year [53% (n=334) vs 17% (n=23), p<0.001]. Unadjusted costs of case patients included in the model and excluding outliers were more expensive than controls [£6,462 (95% CI: 6,128-6,883) vs £4,419 (95% CI: 3,900-5,250), p<0.001]. Cost of hospitalisation within 6 months prior index hospitalisation were also higher in CDI cases [£3,810 (95% CI: 3,300-4,525)] than control patients [£2,532 (95% CI: 1,828-3,618), p=0.032], but costs of hospitalisation within 6 months post index hospitalisation were higher in control patients [£3,487 (95% CI: 2,321-6,440) vs £2,027 (95% CI: 1,720-2,494), p=0.010].

6.4.3.2 Multivariable analysis

Initially, the model included CDI status, age, gender, mode of acquisition, disease and pre-test periods, hospitalisation within 6 months prior to index hospitalisation, mortality during hospitalisation and recruitment as covariates after univariate analysis (Table 6.14). Recruitment variable was included in the initial model as a potential confounder. The process to choose the covariates is shown on Table 6.15 and all variables included in the initial model remained in the final model.

Table 6.13 Patients characteristics of cohort and audit phase I patients

	control (n=132)		case (n=633)		
	n	M (95% CI) F (P)	n	M (95% CI) F (P)	p
Demographics					
Age (years)	132	65 (62-68)	633	74 (73-75)	<0.001
gender (female, %)	132	77 (58.3)	633	367 (58.0)	0.940
IMD (score)	129	39.7 (35.9-43.6)	619	40.8 (39.1-42.4)	0.599
Recruitment (recruits, %)	132	132 (100)	633	217 (34.3)	<0.001
Clinical					
mode of acquisition (HCAI, %)	132	80.0 (60.6)	633	434.0	0.077
Hospitalisation					
LoS (days)[a]	132	21.2 (17.7-26.1)	633	39.1 (36.0-42.8)	<0.001
disease (days)[a]	132	12.5 (10.2-16.8)	633	23.5 (21.5-26.4)	<0.001
pre-test (days)[a]	132	8.6 (6.6-12.4)	633	15.6 (14.0-17.6)	<0.001
LoS before (days)[a]	132	6.4 (4.5-9.8)	633	13.1 (11.5-15.4)	0.003
LoS after (days)[a]	132	12.9 (7.8-29.9)	633	7.6 (6.2-9.2)	0.212
Time to death (days)	23	137 (96-187)	334	72 (62-81)	0.001
Mortality hospitalisation (%)	132	5 (3.8)	633	181 (28.6)	<0.001
4 weeks (%)	132	6 (4.6)	633	155 (24.5)	<0.001
1 year (%)	132	23 (17.4)	633	334 (52.8)	<0.001
Costs index (£)[a]	132	£4,726 (4,045-5,588)	633	£7,710 (7,141-8,467)	<0.001
model (£)[a]	128	£4,419 (3,9001-5,250)	599	£6,462 (6,128-6,883)	<0.001
before (£)[a]	132	£2,532 (1,828-3,618)	633	£3,810 (3,300-4,525)	0.032
after (£)[a]	132	£3,487 (2,321-6,440)	633	£2,027 (1,720-2,494)	0.010

IMD score: index of multiple deprivation score. HCAI: healthcare-associated infection. LoS: length of stay, LoS before: number of hospitalised days 6 months prior to index hospitalisation, LoS after: number of hospitalised days 6 months post to index hospitalisation, Costs index: costs of index hospitalisation, Costs model: hospitalisation costs excluding outliers (5%), Costs before: costs of hospitalisation 6 months prior to index hospitalisation, Costs after: costs of hospitalisation 6 months post to index hospitalisation.

[a] bias corrected and accelerated confidence intervals

Table 6.14 Univariate analysis of cohort and audit phase I patients

	n	β (SE)	expβ (95% CI)	p
recruitment	776	-0.13 (0.08)	0.87 (0.74-1.02)	0.094
CDI status	765	0.49 (0.10)	1.63 (1.63-1.64)	<0.001
age	776	-0.01 (0.00)	0.99 (0.99-1.00)	0.004
gender	776	-0.12 (0.08)	0.88 (0.75-1.04)	0.129
IMD score	757	0.00 (0.00)	1.00 (1.00-1.00)	0.934
mode of acquisition	776	0.76(0.08)	2.15 (1.83-2.52)	<0.001
disease	776	0.02 (0.00)	1.02 (1.01-1.02)	<0.001
pre-test	776	0.02 (0.00)	1.02 (1.02-1.02)	<0.001
LoS before	776	0.00 (0.00)	1.00 (1.00-1.00)	0.984
Mortality hospitalisation	776	-0.65 (0.28)	0.52 (0.30-0.90)	0.021
4 weeks	776	-0.32 (0.10)	0.72 (0.60-0.87)	0.001
1 year	776	-0.05 (0.08)	0.95 (0.81-1.11)	0.529

IMD: index of multiple deprivation score, LoS before: number of hospitalised days within 6 months prior to index hospitalisation.

GLM result of model with poisson distribution and link log is shown on Table 6.16. Presence of toxin (expβ=1.22, 95% CI: 1.21-1.22), recruited patients (expβ=1.07 (1.06-1.07), male patients (expβ=0.94, 95% CI: 0.94-0.95), health-care associated infection (expβ=1.17, 95% CI: 1.16-1.17), increased disease (expβ=1.02, 95% CI: 1.02-1.02) and increased pre-test (expβ=1.02, 95% CI: 1.02-1.02) periods and mortality during hospitalisation (expβ=1.06, 95%: 1.05-1.06) were associated with higher hospitalisation costs. Between 2008 and 2012, adjusted costs of diarrhoea control patients were on average £5,151±3,353 and cost of CDI cases were on average £6,272±4,082 when considering cohort and audit patients.

Table 6.15 Stepwise process to choose the best model of cohot and audit phase I patients

Initial	CDI status, recruitment, age, gender, mode of acquisition, disease, pre-test, LoS before, mortality hospitalisation				
Final	CDI status, recruitment, age, gender, mode of acquisition, disease, pre-test, LoS before, mortality hospitalisation				
	Step	Residual df	AIC	BIC	p
1	Initial	716	997.47	712932.90	

Table 6.16 GLM with family poisson and link log model of cohort and audit phase I

	β (SE)	**expβ (95% CI)**	**p**
CDI status	0.20 (0.00)	1.22 (1.21-1.22)	<0.001
recruitment	0.06 (0.00)	1.07 (1.06-1.07)	<0.001
age	0.00 (0.00)	1.00 (1.00-1.00)	<0.001
gender	-0.06 (0.00)	0.94 (0.94-0.95)	<0.001
IMD	0.00 (0.00)	1.00 (1.00-1.00)	<0.001
mode of acquisition	0.15 (0.00)	1.17 (1.16-1.17)	<0.001
disease	0.02 (0.00)	1.02 (1.02-1.02)	<0.001
pre-test	0.02 (0.00)	1.02 (1.02-1.02)	<0.001
LoS before	0.00 (0.00)	1.00 (1.00-1.00)	<0.001
Mortality hospitalisation	0.05 (0.00)	1.06 (1.05-1.06)	<0.001

IMD: index of multiple deprivation, LoS before: number of hospitalised days within 6 months prior to index hospitalisation.

Table 6.17 Modified Park test to assess choice of family for GLM model of cohort and audit phase I

	Chi2	**p**
Gaussian	17.24	<0.001
Poisson	0.431	0.512
Gamma	29.86	<0.001
Inverse Gaussian	105.53	<0.001

Modified Park test (Table 6.17) showed poisson distribution as the recommended family for GLM. Poisson distribution shows a variance proportional to mean. Both graphs to assess goodness of model fit (Figure 6.5 and 6.6) confirmed the normality and homoscedasticity of residuals.

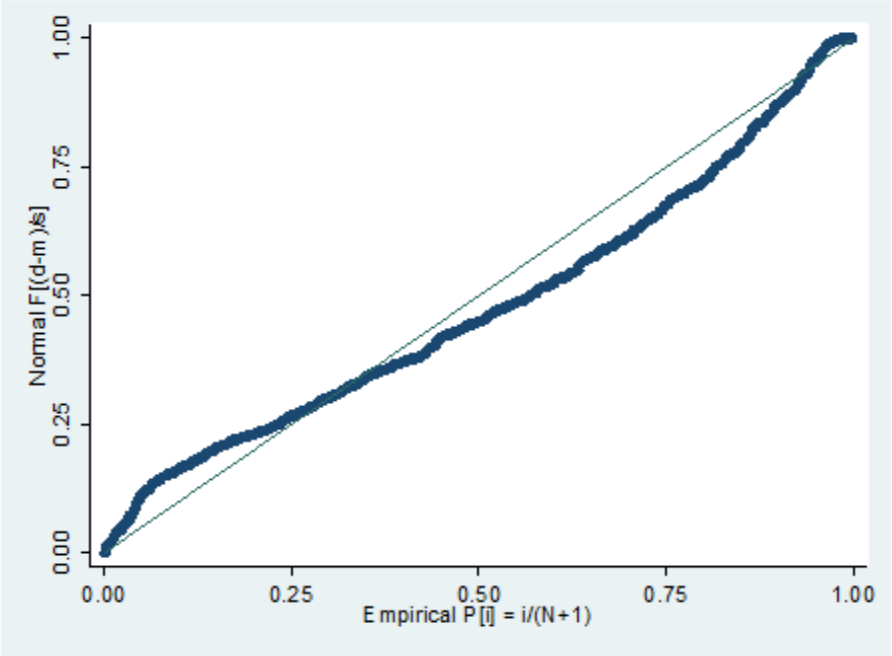

Figure 6.5 Plot of probability of standard deviance residual distribution against costs of cohort and audit phase I model

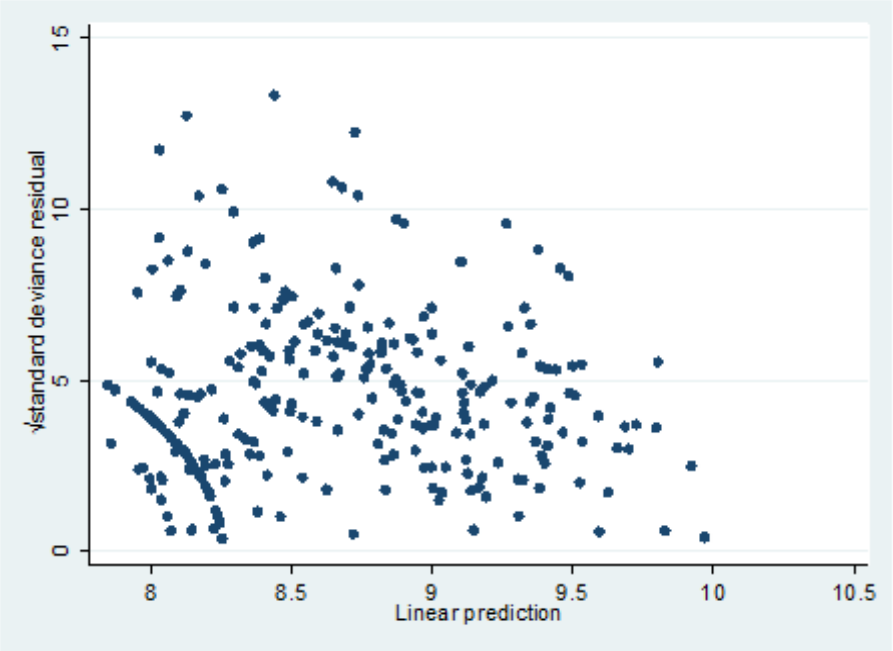

Figure 6.6 Plot of square root of standard deviance residual against linear prediction of cohort and audit phase I model

6.4.4 Phase II cohort and audit patients

6.4.4.1 Patient characteristics

As cohort and audit patients without hospitalisation data during the index episode were not considered, 204 case patients, 383 carrier patients and 3,703 control patients were included in this analysis. Case group was older than carrier and control groups [70 (95% CI: 68-73) vs 66 (95% CI: 64-67) vs 64 years (95% CI: 64-65), p<0.001) and most of the patients were female. Case and carrier patients stayed hospitalised similar time considering the whole hospitalisation and the period post *C. difficile* test but more time comparing to control patients [LoS 27.8 (95% CI: 24.1-32.8) vs 29.1 (95% CI: 25.6-33.9) vs 18.3 days (95% CI: 17.6-19.2), p<0.001; disease period was 17.1 (95% CI: 14.6-20.2) vs 15.8 (95% CI: 14.0-18.8) vs 11.4 days (95% CI:11.0-12.0), p<0.001]. Considering the hospitalised time before toxin test and hospitalised period within 6 months prior to index hospitalisation carriers stayed more days in hospital than CDI cases and control patients [pre-test 13.3 (95% CI: 10.9-16.8) vs 10.8 (95% CI: 8.6-14.2) vs 6.9 days (95% CI: 6.5-7.5), p<0.001); Los before 12.7 (95% CI: 10.3-15.7) vs 11.4 (95% CI: 9.0-14.5) vs 5.0 days (95% CI: 4.6-5.4), p<0.001], but hospitalisation period post index hospitalisation was higher in potential carrier patients and similar comparing control patients and CDI cases [Los after 13.9 (95% CI: 11.0-19.7) vs 7.0 (95% CI: 6.4-7.7) vs 6.2 95% CI: 4.2-10.7), p<0.011]. Mortality rate was higher in case groups in all scenarios, during hospitalisation [20% (n=40) vs 13% (n=51) vs 9% (n=332), p<0.001]; within 4 weeks [20% (n=41) vs 15% (n=59) vs 11% (n=399), p<0.001]; and within 1 year [53% (n=107) vs 40% (n=153) vs 28%

Table 6.18 Patients characteristics of cohort and audit phase II

	control (n=3,703)		carrier (n=383)		case (n=204)		
	n	M (95% CI) / F (P)	n	M (95% CI) / F (P)	n	M (95% CI) / F (P)	p
Demographics							
Age (years)	3,703	64 (64-65)	383	66 (64-67)	204	70 (68-73)	<0.001
gender (female, %)	3,703	1,896 (51.2)	383	215 (56.1)	204	115 (56.4)	0.078
IMD (score)	3,604	40.1 (39.4-40.8)	375	40.6 (38.6-42.8)	195	40.3 (37.1-43.4)	0.928
Clinical							
mode of acquisition (HCAI, %)	3,703	1,735 (46.8)	383	211.0 (55.1)	204	103.0 (50.5)	0.006
Hospitalisation							
LoS (days)[a]	3,703	18.3 (17.6-19.2)	383	29.1 (25.6-33.8)	204	27.8 (24.1-32.8)	<0.001
disease (days)[a]	3,703	11.4 (11.0-12.0)	383	15.8 (14.0-18.8)	204	17.1 (14.6-20.2)	<0.001
pre-test (days)[a]	3,703	6.9 (6.5-7.5)	383	13.3 (10.9-16.8)	204	10.8 (8.6-14.2)	<0.001
LoS before (days)[a]	3,703	5.0 (4.6-5.4)	383	12.7 (10-15)	204	11.4 (9.0-14.5)	<0.001
LoS after (days)[a]	3,703	7.0 (6.4-7.7)	383	13.9 (11.0-19.7)	204	6.2 (4.2-10.7)	<0.001
Time to death (days)	1,039	92 (86-98)	153	87 (75-102)	107	88 (71-108)	0.760
Mortality hospitalisation (%)	3,703	332 (9.0)	383	51 (13.3)	204	40 (19.6)	<0.001
4 weeks (%)	3,703	399 (10.8)	383	59 (15.4)	204	41 (20.1)	<0.001
1 year (%)	3,703	1039 (28.1)	383	153 (40.0)	204	107 (52.5)	<0.001
Costs index (£)[a]	3,703	£6,332 (6,039-6,442)	383	£8,091 (7,120-9,228)	204	£7,130 (6,338-8,301)	<0.001
model (£)[a]	3,396	£4,128 (4,023-4,246)	349	£5,471 (5,075-5,980)	190	£6,015 (5,544-6,592)	<0.001
before (£)[a]	3,703	£2,261 (2,108-2,428)	383	£4,865 (4,001-6,014)	204	£4,017 (3,275-5,324)	<0.001
after (£)[a]	3,703	£2,413 (2,248-2,614)	383	£4,041 (3,209-5,324)	204	£2,248 (1,694-3,187)	0.004
Medicines							
Fidaxomicin	3,703	0	383	128 (33.4)	204	130 (63.7)	<0.001

IMD score: index of multiple deprivation score, HCAI: healthcare-associated infection, LoS: length of stay, LoS before: number of hospitalised days 6 months prior to index hospitalisation, LoS after: number of hospitalised days 6 months post to index hospitalisation, Costs index: costs of index hospitalisation, Costs model: hospitalisation costs excluding outliers (5%), Costs before: costs of hospitalisation 6 months prior to index hospitalisation, Costs after: costs of hospitalisation 6 months post to index hospitalisation. [a] bias corrected and accelerated confidence intervals.

(n=1,039), p<0.001]. Unadjusted hospitalisation costs omitting outliers, were more expensive in case group compared to potential carrier patients and diarrhoea controls [£6,015 (95% CI: 5,544-6,592) vs £5,471 (5,075-5,980) vs £4,128 (95% CI: 4,023-4,246), p<0.001]. Cost of hospitalisation 6 months prior index episode were higher for carrier patients [£4,865 (95% CI: 4,001-6,014)] compared to CDI cases [£4,017 (95% CI: 3,275-5,324)] and controls [£2,261 (95% CI: 2,108-2,428), p<0.001] and costs of hospitalisation 6 months post index episode were similar in CDI cases [£2,248 (95% CI: 1,694-3,187)] and diarrhoea controls [£2,413 (95% CI: 2,248-2,614)] but higher for potential carrier group [£4,041 (95% CI: 3,209-5,324), p=0.004]. All characteristics are shown on Table 6.18.

6.4.4.2 Multivariable analysis

The initial model included CDI status, disease and pre-test periods, hospitalisation time prior to index hospitalisation, age, gender, mortality during hospitalisation, IMD score, mode of acquisition and recruitment variables. Univariate analysis was performed to help this choice (Table 6.19). Only recruitment variable was dropped from the initial model (Table 6.20).

Table 6.19 Univarite analysis of cohort and audit phase II patients

	n	β (SE)	expβ (95% CI)	p
recruitment	4,358	0.09 (0.10)	1.09 (0.90-1.33)	0.387
CDI status	4,290	0.12 (0.05)	1.13 (1.03-1.23)	0.010
age	4,358	-0.01 (0.00)	0.99 (0.99-1.00)	<0.001
gender	4,358	-0.18 (0.04)	0.84 (0.77-0.91)	<0.001
IMD score	4,240	-0.01 (0.00)	0.99 (0.99-0.99)	<0.001
mode of acquisition	4,344	1.00 (0.04)	2.73 (2.55-2.93)	<0.001
disease	4,344	0.03 (0.00)	1.03 (1.02-1.03)	<0.001
pre-test	4,344	0.03 (0.00)	1.04 (1.03-1.04)	<0.001
LoS before	4,358	0.00 (0.00)	1.00 (1.00-1.00)	0.920
mortality hospitalisation	4,358	-0.50 (0.20)	0.61 (0.41-0.90)	0.014
4 weeks	4,358	-0.23 (0.07)	0.79 (0.69-0.91)	0.001
1 year	4,358	0.00 (0.05)	1.00 (0.91-1.10)	0.975
Fidaxomicin	4,358	0.26 (0.09)	1.30 (1.08-1.56)	0.006

IMD score: index of multiple deprivation score, LoS before: number of hospitalised days within 6 months prior to index hospitalisation.

Table 6.20 Stepwise process to choose the best model of cohot and audit phase II patients

Initial	CDI status, recruitment, age, gender, IMD, mode of acquisition, disease, pre-test, LoS before, mortality hospitalisation
Final	CDI status, age, gender, IMD, mode of acquisition, disease, pre-test, LoS before, mortality hospitalisation

	Step	Residual df	AIC	BIC	p
1	Initial	3,923	952.19	3675241	
2	recruitment	3,924	952.19	3675235	0.171

According to the best model chosen (Table 6.21) with poisson distribution and link log, male patients (expβ=0.96, 95% CI: 0.96-0.96), healthcare-associated infection (expβ=1.10, 95% CI: 1.10-1.10), increased disease (expβ=1.02, 95% CI: 1.02-1.02) and pre-test (expβ=1.02, 95% CI: 1.02-1.02) periods and mortality during hospitalisation (expβ=1.10, 95% CI: 1.10-1.10) were associated with hospitalisation costs of cohort and audit phase II patients. Also, potential carrier patients (expβ=0.98, 95% CI: 0.97-0.98) and CDI cases (expβ=0.95, 95% CI: 0.94-0.95) not treated with FDX were negatively

associated with costs and potential carrier (expβ=1.28, 95% CI: 1.28-1.28) and cases (expβ=1.34, 95% CI: 1.34-1.34) who received FDX had a positive association.

Table 6.21 GLM with family poisson and link log model of cohort and audit phase II

	β (SE)	expβ (95% CI)	p
NF - GDH+/TOX-[a]	-0.02 (0.00)	0.98 (0.97-0.98)	<0.001
F - GDH+/TOX-[a]	0.25 (0.00)	1.28 (1.28-1.28)	<0.001
NF - GDH+/TOX+[b]	-0.05 (0.00)	0.95 (0.94-0.95)	<0.001
F - GDH+/TOX+[b]	0.29 (0.00)	1.34 (1.34-1.34)	<0.001
age	0.00 (0.00)	1.00 (1.00-1.00)	<0.001
gender	-0.04 (0.00)	0.96 (0.96-0.96)	<0.001
IMD score	-0.00 (0.00)	1.00 (1.00-1.00)	<0.001
mode of acquisition	0.16 (0.00)	1.17 (1.17-1.17)	<0.001
disease	0.02 (0.00)	1.02 (1.02-1.02)	<0.001
pre-test	0.02 (0.00)	1.02 (1.02-1.02)	<0.001
LoS before	-0.00 (0.00)	1.00 (1.00-.100)	<0.001
Mortality hospitalisation	0.09 (0.00)	1.10 (1.10-1.10)	<0.001

NF: no fidaxomicin treatment, F: Fidaxomicin treatment, IMD: index of multiple deprivation score, LoS before: number of hospitalised days within 6 months prior to index hospitalisation.
[a] carrier patients [b] CDI case patients

Adding audit patients to the analysis, diarrhoea control patients had adjusted hospitalisation costs on average of £4,251±2,880, potential carrier patients not treated with FDX of £4,145±2,808, potential carrier patients treated with FDX of £5,448±3,692, CDI cases that did not received FDX treatment of £4,027±2,728 and CDI who received FDX of £5,694±3,859 between 2012 and 2016. Chi-square of modified Park test (Table 6.22) showed poisson distribution as the best choice. Although there are still some outliers in the model, both graphs to assess goodness of model fit (Figure 6.7 and 6.8) confirmed the normality and homoscedasticity of the residuals. We chose not to remove more outliers as high cost patients are common and should be

considered in the model. Table 6.23 summarises all hospitalisation costs found in all 4 models.

Table 6.22 Modified Park test to assess choice of family for GLM model of cohort and audit phase II

	Chi2	p
Gaussian	172.30	<0.001
Poisson	8.88	0.003
Gamma	51.37	<0.001
Inverse Gaussian	172.30	<0.001

Table 6.23 Summary of mean adjusted costs for model performed

Model	Patients	CDI cases	Potential carriers	Controls	Additional costs
1	Cohort phase I	£5,761	.	£4,924	£837
2	Cohort phase II (no fidaxomicin)	£5,011	£4,504	£4,227	£784
3	Cohort phase II (fidaxomicin)	£6,355	£5,746	£4,227	£2,128
3	Cohort and audit phase I	£6,272	.	£5,151	£1,121
4	Cohort and audit phase II (no fidaxomicin)	£4,027	£4,145	£4,251	-£224
4	Cohort and audit phase II (fidaxomicin)	£5,694	£5,448	£4,251	£1,443

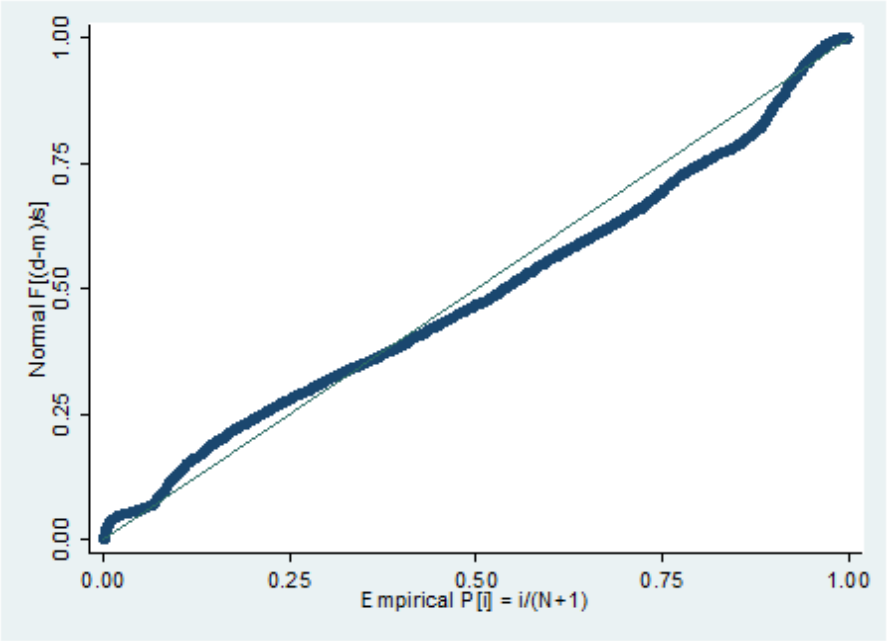

Figure 6.7 Plot of probability of standard deviance residual distribution against costs of cohort and audit phase II model

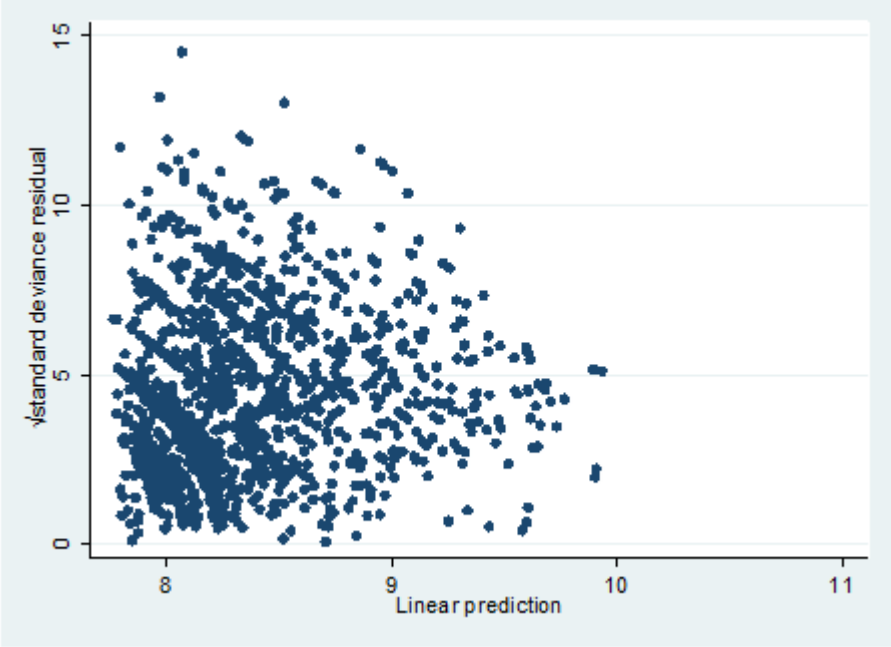

Figure 6.8 Plot of square root of standard deviance residual against linear prediction of cohort and audit phase II model

6.4.5 Costs per year

Using additional costs of CDI cases from the last two models, considering FDX as first-line treatment and data from PHE (PHE, 2017), we could estimate that between April 2007 and March 2016 RLBUHT spent additional £1.6 million, Merseyside hospitals £6 million and UK hospitals £124 million to treat 1,423, 5,182 and 106,098 CDI patients (Table 6.24).

Table 6.24 Number of CDI cases and additional costs of CDI at RLBUHT, Merseyside hospitals and England hospitals between 2007 and 2016.

	RLBUHT		**Merseyside**		**England**	
Year	n	costs	n	costs	n	Costs
2007	512	£573,952	1,628	£1,824,988	33,434	£37,479,514
2008	347	£388,987	1,185	£1,328,385	19,927	£22,338,167
2009	220	£246,620	688	£771,248	13,220	£14,819,620
2010	105	£117,705	457	£512,297	10,417	£11,677,457
2011	64	£71,744	308	£345,268	7,689	£8,619,369
2012	53	£59,413	224	£251,104	5,980	£6,703,580
2013	50	£72,150	234	£337,662	5,034	£7,264,062
2014	43	£62,049	231	£333,333	5,233	£7,551,219
2015	29	£41,847	227	£327,561	5,164	£7,451,652
Total	1,423	£1,634,467	5,182	£6,031,846	106,098	£123,904,640

RLBUHT: Royal Liverpool and Broadgreen University Hospitals Trust

6.5 Discussion

We estimated costs of cohort and audit patients tested positive or negative for *C. difficile* toxin during 2008-2012 and cohort and audit CDI case patients, patients tested positive for GDH but negative for *C. difficile* toxin and control patients during 2012 and 2016. During the first phase, CDI cases mean unadjusted costs were £6,246 and control patients mean costs were £4,419, in the second phase, hospitalisation costs of CDI cases were £6,015, carrier

patients £5,741 and control patients £4,128. GLM models showed that during phase I, cases patients were between 17% (£5,761 vs £4,924 for cohort patients) and 22% (£6,272 vs £5,151 for audit and cohort patients) more expensive than controls. During phase II carrier patients that received standard treatment were 2% less expensive (£4,145 vs £4,251 for audit and cohort patients) to 7% more expensive (£4,504 vs £4,227 for cohort patients) compared to controls, but potential carrier patients treated with FDX were 28% (£5,448 vs £4,251 for audit and cohort patients) to 36% (£5,746 vs £4,227 for cohort patients) more expensive than control patients. Case patients not treated with FDX were 5% less expensive (£4,027 vs £4,251 for cohort and audit patients) and 19% more expensive (£5,011 vs £4,227 for cohort patients) compared to diarrhoea controls. Lastly, patients with a positive toxin test and treated with FDX had costs 34% (£5,694 vs £4,251 for audit and cohort patients) and 51% (£6,355 vs £4,227 for cohort patients) higher than control patients. Although CDI cases patients have incurred higher costs compared to other patients, it was not possible to find a trend when considering different recruitment groups (cohort or audit) and different phases (I and II). However, when audit patients were included, additional costs were higher than only cohort patients and during phase II costs of diarrhoea control patients were on average £800 lower compared to phase I.

Total costs of CDI treatment were previously assessed and were between $9,822 and $12,854 (equivalent to £5,756 and £7,532 in 2008) compared to $6,950 and $9,008 (equivalent to £4,073 and 5,279 in 2008) for controls in 13 CDI economic studies considering the currency 2008 US dollars (Ghantoji et al., 2010). In 2014, another review (Gabriel and Beriot-Mathiot, 2014) was

published on hospitalisation stay and costs attributable to CDI where seven studies were identified and mean costs for healthcare associated infections were between $2,454 and $29,000 in 2013 US dollars (equivalent to £1,562 to £18,469 in 2013). Also, a recent systematic review by Nanwa et al (Nanwa et al., 2015) has identified 45 cost of illness (COI) studies from seven different countries where mean attributable costs in 2014 US dollars per patient ranged from $8,911 to $30,049 (equivalent to £5,458 to £18,408 in 2014). The only UK study published and identified in all these three reviews was conducted in 1996 including 50 CDI cases and 92 control patients and case patients were around £4,000 more expensive than controls (Wilcox et al., 1996). In 2017 another study was published with a UK cohort and median costs for recurrence cases was £7,539 and for first cases was £6,294 in GBP 2014. Both UK studies did not use the same methodology to calculate costs as our study. The first study only considered the average difference between LoS to calculate costs, while the second study calculate costs based on hospital bed days, use of medicines, laboratory tests, procedures and intravenous support in 28 days of hospitalisation.

Despite costs were statistically significant higher in case than control patients in our study and in the literature, studies have different methodologies to perform economic analyses, different currency and cost calculation and also different population and samples. Thus, the average costs are just applicable for the respective study and it is not possible to make meaningful comparisons between studies and countries (Gabriel and Beriot-Mathiot, 2014, Nanwa et al., 2015). For more reliability and reproducibility, Gabriel and Nanwa studies

(Nanwa et al., 2015, Gabriel and Beriot-Mathiot, 2014) suggest the development of guidelines and the use of standard COI methodology.

LoS is also a common outcome variable in COI studies and usually strongly related to costs of hospitalisation. In our study, during phase I mean LoS of CDI cases was 39 days while mean LoS of control patients was 21 days ($p<0.001$). On phase II, mean LoS of CDI cases and control patients were lower compared to phase I and was around 28 days and 18 days, respectively. Potential carrier patients stayed hospitalised around 29 days. Similar time of hospitalisation were found by Ghantoji et al (Ghantoji et al., 2010) where mean LoS for cases was 24 days (10-46 days) and for controls was 11 days (4-25 days). In the Gabriel et al review (Gabriel and Beriot-Mathiot, 2014) LoS were lower and ranged between 3 and 21 days for CDI cases. In the UK study, mean LoS was higher for both cases (46 days) and controls (25 days) in 1994/1995 (Wilcox et al., 1996) and in 2013/2014 the median LoS was 21 days for recurrent cases and 16 days for first cases (Wilcox, 2017). The decrease in hospitalisation period showed in phase I groups compared to phase II groups could be related to the transition from an epidemic period to an endemic period, to successful prevention and controlling measures implemented by the hospital during the years, to the implementation of a new diagnostic algorithm in 2012 or to the use of FDX as CDI treatment since 2011. LoS is an important marker to be followed and assessed as it is not only a consequence of medical conditions but also a risk factor for the development of several diseases including CDI, as it will determine time of exposure to microorganisms (Cohen et al., 2010).

The cost of FDX treatment was added to the hospitalisation costs and showed an increase of 27 to 41% in CDI case costs and an increase of 28 to 31% in potential carrier group. Disease and pre-test periods and healthcare-associated infection were predictors of costs in the models, for every hospitalised day during these periods the cost increased 2% and healthcare-associated infections increased costs between 17 to 30% compared to community-acquired infection. When we included audit patients in the model more variables became predictors of costs, as gender, mortality during hospitalisation, age, hospitalisation within 6 months prior to index hospitalisation and IMD score. Male patients were between 4 to 6% more expensive than females, patients who died during hospitalisation were 6 to 10% more expensive, age, IMD score and hospitalisation prior index hospitalisation changed an insignificant amount to the costs.

C. difficile is still a problem for healthcare settings and systems because of its burden and the difficulty to treat (Trafford, 2017). Different studies found that annual costs of CDI cases in the US were around $1.1 billion (equivalent to £680 million) in 1999 (Kyne et al., 2002), $496 million (equivalent to £270 million) (McGlone et al., 2012) and $4.8 billion (equivalent to £2.6 billion) in 2008 (Dubberke and Wertheimer, 2009) and $6.3 billion (equivalent to £4.1 billion) in 2015 (Zhang et al., 2016). Annual costs to Canadian Society in 2012 was estimated in CAD$280 million (equivalent to £177 million) (Levy et al., 2015). European Hospital and Healthcare Federation (HOPE, 2013) estimated a total cost of €3 billion (equivalent to £2 billion) in 2006 and around €3.7 billion (equivalent to £3.1 billion) in 2013 for the European Union. Estimated annual costs in the UK decreased from £210 million in 2007/2008 to £29 million in

2016/2017. Although the number of cases has been decreasing during the years and it may have reached a constant incidence in the UK and Merseyside hospitals in the last 3 years, RLBUHT on the other hand remains decreasing CDI cases and in 2016/2017 was considerably below target (29 of 44) confirming the effectiveness of intervention measures adopted by the site.

This study has some limitations. Firstly, as with the majority of COI studies of CDI, our study does not calculate indirect costs. Most of the studies have considered only direct costs and include mainly medical costs, such as hospitalisation, health care professionals, procedures, laboratory test and medicines. On Nanwa review (Nanwa et al., 2015), only one identified study has calculated indirect costs and no indirect cost analysis was identified in Ghatoji review (Ghantoji et al., 2010).

Also, it was not possible to collect patient-level information and costing system (PLICS) data as we could not have access to the database. Thus, we have used HRG codes and national tariff payment system that is not accurate as it does not represent a real cost of each patient during hospitalisation period, but it is an average cost of a code given to the patient according to all diagnostic and procedures performed in this period not considering the high cost of FDX, for example. Moreover, not all patients had available hospitalisation information and for this reason they were excluded from the analysis, however they might have not incurred costs for the setting during the episode as there are no register of their hospitalisations on the information department system.

Finally, our findings are not applicable to other hospitals outside the UK, as costs of hospitalisation are entirely related to the healthcare system. Thus, a

standardised method should be developed and implemented to allow comparison of different studies in different hospitals and countries.

In conclusion, hospitalisation costs were related to CDI status, patients positive for *C. difficile* toxin and patients positive for GDH but negative for *C. difficile* toxin were around 35% and 10% more expensive than control patients, respectively. Although we can find similar results in the literature, it is not possible to compare studies as the methodologies were different. Also, it is important to highlight that our study use hospitalisation costs obtained through codes of diagnostic and procedures performed and did not consider non-medical and indirect costs.

Chapter 7

Final discussion

CDI is still a challenge for healthcare settings and healthcare systems. Although the number of cases in the UK has been decreasing over the years since the well-publicised outbreaks, there is still a need to understand the disease. This thesis has considered different clinical and economic approaches to CDI, including clinical outcomes, diagnosis algorithms and contribution of patients with discordant test results, stratification of disease using a biomarker, cost-effectiveness of interventional measures and costs of hospitalisation.

The outbreak caused by the RT027 strain put healthcare settings on alert. In 2008, we started recruiting CDI case and diarrhoea control patients to better understand the disease (Chapter 2). The recruitment occurred during epidemic and endemic phases. This thesis has brought together information from a large number of patients, and supplemented this with a clinical audit to increase the number of patients studied and information available. The audit has confirmed that inclusion and exclusion criteria utilised for study recruitment produced a cohort which was not representative of overall patients tested for *C. difficile* in the setting. The most important aspect was that the patients in the audit were more debilitated and thus were not recruited largely because of refusal. Mortality was higher in non-recruited patients at all time points and reached more than 25% during hospitalisation and within 4 weeks and more than 50% within 1 year after CDI diagnosis. Thus, the inclusion of patients from the clinical audit could also increase the strength of the study by minimising the selection bias of the cohort recruitment.

Diagnostic methods have been improving and becoming more specific and sensitive for identifying CDI cases. In this thesis, we have shown that the

introduction of a new assay was cost-saving considering the unnecessary use of FDX to treat non-toxigenic (PCR-) patients (Chapter 3). This is an important finding as there is a debate about the best method to diagnose CDI. Whist UK (PHE, 2013) and Europe (Crobach et al., 2016) recommend the use of a 2-step algorithm, in the US NAAT has been commonly used as a single test (Fang et al., 2017). Both approaches have advantages and disadvantages and a consensus may be difficult to achieve, and choice is likely to be dominated by local expertise, availability of equipment and financial resources.

Clinical outcomes of patients were assessed (Chapter 3); carrier patients (GDH+/TOX-/PCR+) and case patients had similar clinical outcomes, such as mortality rates, time to discharge and costs in the multivariable analysis considering only toxigenic strains. In these models, the presence of a toxin positive result was only significant for CDI severity. When considering all patients, carriers and cases had worse outcomes when compared to diarrhoea control patients.

Currently, no prognostic test for the prediction of clinical outcomes in CDI patients is available. This is important mainly to avoid recurrence, as recurrence rate is about 25% after the first episode. Although some studies (Rao et al., 2013, Dazley et al., 2015) have been published showing a positive association between PCT levels and CDI severity, this is the first study, using a bigger sample size, to assess mortality rates, recurrence rates and time to discharge in CDI patients and diarrhoea control patients (Chapter 4). High levels of serum PCT were associated with CDI severity, long-term mortality and CDI diagnosis and were associated with increased risk of delayed discharge in some scenarios. However, a positive result for TOX was not a

predictor of any outcome when considering only PCT tests performed within 3 days after toxin test.

One of the main challenges is to control and prevent the transmission of bacteria. Interventional measures are mainly focused on reducing the spread of the bacteria and reducing the chance of developing the disease through the use of antibiotics. Nevertheless, the majority of studies included in the systematic review (Chapter 5) were based on treatment of disease and prevention of recurrences. Screening during admission, vaccination, treatment with FDX and FMT were interventions identified as cost-effective measures for CDI considering papers published until 2015.

CDI poses a significant financial burden for healthcare systems worldwide. In our setting, costs of hospitalisation, as expected, were higher for CDI patients compared to diarrhoea control patients (Chapter 6). Recruitment status (cohort or audit) was indifferent but epidemic phase (phase I) had higher costs than endemic phase (phase II) patients, even with the addition of FDX costs for phase II patients treated with this antibiotic. Predictors of costs were duration of disease and pre-test periods and healthcare-associated infection. Gender, age, mortality during hospitalisation, time of hospitalisation within 6 months before index hospitalisation and IMD score were also significant predictors when including non-recruited patients. Last year around £86,000 was spent to treat 29 cases, a reduction of more than 95% compared with the peak of the outbreak.

As national and international guidelines have been updated according to epidemiological circumstances and healthcare settings have subsequently

altered their policies based on these recommendations, some of these changes have affected the work described in the chapters, such as group categorisation based on diagnostic tests, and use and contribution of antibiotics to clinical outcomes. The real hospitalisation cost of each patient (PLICS) was not possible to obtain and costs of hospitalisation used for all analysis were based on HRG codes and national tariff. This value may not reflect a patient infected by *C. difficile*, as some interventions (antibiotics and isolation ward) are more expensive than for a standard patient or patients with diarrhoea. Also, 2016/2017 national tariff prices were based on costs from the 2011/2012 reference costs (NHS England, 2016). These costs are collected every financial year and refer to the average unit cost of healthcare provided and are adjusted according to the resource allocations based on differences between geographical locations of healthcare providers to compose the national tariff (NHS England, 2016). Moreover, not all patients recruited as a CDI case had an International Classification of Diseases (ICD)-10 code for CDI, and costs were associated with the whole hospitalisation period and not only with the CDI episode.

Studies conducted using the same phase I cohort found that low levels of serum mannose-binding lectin was associated with CDI recurrence (Swale et al., 2014a), but did not find a clinical association between levels of the biomarkers faecal calprotectin and lactoferrin and CDI severity (Swale et al., 2014b) and between faecal interleukin 8 and CDI recurrence (Miyajima et al., 2014). Moreover, when considering patients infected by RT027 strain between 2008 and 2010, genome-based infection tracking was considered a relevant tool to monitor persistence and transmission of the bacteria (Kumar et al.,

2016). This same tool was used in other hospitals and confirmed its relevance to infection control when assessing *C. difficile* transmission and new cases linked to previous ones (Eyre et al., 2017).

A study published this year (Dingle et al., 2017) found that the antibiotic stewardship introduced to restrict the use of fluoroquinolones in 2007 was a central strategy to decrease the number of HCAI and CAI cases after the outbreak in England. This decrease was higher than 75% considering all cases, however the proportion of CAI cases has been increasing during the years (Gupta and Khanna, 2014) and became higher than HCAI in England (PHE, 2017). Moreover, genome-sequencing studies have shown that only a low rate of cases were associated with a previous case (Eyre et al., 2017, Kumar et al., 2016), indicating that the problem may be wider. Airborne transmission, water and food contamination and asymptomatic patients are described in the literature as potential sources of bacteria (Gupta and Khanna, 2014). Thus, studies on interventions to control and prevent transmission through these sources should also be undertaken. Potential carriers and asymptomatic patients may also play an important role on the spread of bacteria and for this reason the identification of those patients may be considered. Indeed, a CEA included in the systematic review concluded that the screening of all patients during hospital admission was a cost-effective strategy (Bartsch et al., 2012a).

The pattern of FDX use is variable and each hospital can adopt a different protocol for its use (Goldenberg et al., 2016). Its use as a first line treatment for all CDI cases was a response from RLBUHT to *C. difficile* targets and financial penalties implemented by the UK government as an alternative to

control CDI cases (Walker et al., 2008). Considering the recurrence rates and short-term mortality, FDX showed superior results when used as a first-line treatment compared to the use in selected episodes or recurrences (Goldenberg et al., 2016). Financially, it was a good choice as penalties could cost around £50,000 for every case exceeding targets, and this antibiotic is known to decrease recurrence rates, however, its use regardless of the CDI severity or presence of toxin positive result is not recommended by PHE (PHE, 2013). Although CEA studies selected in the systematic review have not shown its cost-effectiveness as a first option (Wagner et al., 2014, Bartsch et al., 2013), some recent studies have shown positive results. For instance, it was cost-saving when considering 65 patients treated in a London hospital (Nesnas et al., 2014), and in a study comparing 49 patients treated with FDX with 46 patients treated with VAN in the US (Gallagher et al., 2015). Additionally, it has been shown to be cost-saving and cost-effective in patients with increased recurrence risk (Watt et al., 2016).

New findings and advances to improve CDI and its implications have been published worldwide. Candidate vaccines have shown promising results in preventing CDI. Pfizer (PF-06425090) and Valneva (VLA84) are developing vaccines, both currently in phase 3 trials, with estimated study completion dates later than 2020 (Pfizer, 2017, Kociolek and Shulman, 2017), however, a clinical trial on toxoid vaccine H-030-012 (Cdiffense) from Sanofi Pasteur was stopped as an interim trial concluded that the probability for success would be low (FierceFarma, 2017). Although results and information are still not available, a simulation model found that vaccination was a cost-effective strategy to prevent recurrence when costs were below $800 and efficacy

above 50% (Lee et al., 2010). Other antibiotics and biologic therapies such as cadazolid, ridinilazole and bezloxumab are also being studied. Cadazolid is currently in phase 3 trial with estimated study completion in 2021 (Actelion, 2017), patients treated with this antibiotic in phase 2 showed low baseline minimum inhibitory concentration (MIC) values compared to VAN and was similar compared to FDX regardless of the clinical outcomes or *C. difficile* strain (Gerding et al., 2016). Recently, Summit Therapeutics was awarded with $62 million to develop ridinilazole (SMT19969) (Alliance News, 2017), an antibiotic in phase 2 trials that has already been shown to be superior to VAN when considering clinical cure and recurrence within 30 days (Vickers et al., 2017). Bezlotoxumab, a human monoclonal antibody, on the other hand, was approved and launched in 2017 to prevent CDI recurrence when used in combination with standard antibiotic treatment. NICE has already published an evidence summary of the medicine (NICE, 2017) and the average cost of a single intravenous dose is £2,470. Recent advances have also been made in CDI diagnosis (Pollock, 2016), ultrasensitive assays have shown promising results as they can diagnose CDI with high sensitivity and specificity and could also have prognostic value (Song et al., 2015, Chromy et al., 2017). A CEA study showed that it was more cost-effective to invest in prevention by isolating patients than to invest in a more expensive diagnostic method by exchanging a two-step algorithm by a PCR assay considered the gold standard in this study (Schechner et al., 2017).

There is a need for more studies to confirm the results found in this thesis. PCT measures should be performed at different time points to better understand PCT kinetics in CDI and, in the future, implement it as a routine

test to early stratification and prognosis of the disease if found to be clinically effective. The use of PLICS data can provide a more accurate and reliable cost of hospitalisation for each patient and comparison between CDI cases and diarrhoea controls may become more meaningful. Indirect costs should also be part of the calculation. A comparison between costs obtained by HRG codes and PLICS data may be useful to validate our results and also to validate this first method called payment by results implemented in the last decade that uses HRG codes to pay NHS Trusts for each hospitalisation (Appleby et al., 2012). Economic evaluation studies on intervention measures, other than the use of antibiotics, should be conducted as prevention and control are still the main and more important strategy to avoid new cases. Moreover, a CEA could be conducted using cost and quality of life data obtained from phase II cohort patients to adapt models and results to the local reality. Recently, a health-related quality of life questionnaire (HROOL) specific for CDI (Cdiff32) was developed and validated (Garey et al., 2016) and its use can overcome the limitation of published papers that used utility information for non-infectious diarrhoea.

Furthermore, patient care and quality of life should of course be a priority for the research being undertaken. A study (Madeo and Boyack, 2010) interviewed some patients to identify their experiences and perceptions, and found that the main points are negative physical effects including pain, loss of appetite and energy, inability to control the bowel function caused by diarrhoea, understanding of illness as levels of understanding varied among patients and some of them were concern about the disease, and social and mental consequences including anxiety, depression, mood changes and

issues with privacy and dignity. In addition, many patients are now sharing their experiences in online forums for this disease, it is possible to comprehend their frustrations and insecurities, as it is a debilitating disease, bringing a feeling of shame and embarrassment and having an impact on the physical, psychological, social and financial aspects of life. It is important to give patients all the information and support they need. Patient and public education is another strategy to be considered by healthcare settings and healthcare systems as a lack of knowledge and information could be a reason for transmission of not only *C. difficile* but also of other infectious disease. A recently published study concluded that there is a demand for videos about CDI, mainly on modes of transmission and prevention, also, governmental agencies should use social media to inform and access a higher percentage of the population (Basch et al., 2017). CDI is still considered a problem worldwide, and research on diagnosis, treatment and prevention is important to reduce the morbidity and mortality associated with the disease, and its associated costs.

Appendices

Contents

1: Consent form of individual susceptibility to *Clostridium difficile* infection (CDI) study

Version 6, 13th November 2014

The Royal Liverpool and Broadgreen University Hospitals NHS Trust

Prescot Street
Liverpool
L7 8XP
Tel no: 0151 706 2000

Consent Form – Case Group

Individual susceptibility to *Clostridium difficile* infection (CDI)

Name of principal investigator: Prof M Pirmohamed

Please initial each box before signing the form:

1. I confirm that I have read the understood the information sheet dated 13th November 2014 (Version 6) for the above study ☐
2. I have had the opportunity to discuss the research and ask questions ☐
3. I understand that my participation is voluntary and I may withdraw up until the time that my blood/urine/faeces samples can still be identified ☐
4. I agree to have skin swabs taken as per NHS trust protocol ☐
5. I understand that the results will not be added to my medical records ☐
6. I give permission to the researchers to have access to my medical records and to contact my GP to obtain information relevant to this study ☐
7. I agree to have blood samples taken for tests on genes and other factors that may be involved in determining how I respond to certain antibiotics and the treatment. ☐
8. I understand that my samples will be anonymised and will be stored, and it will not be possible to trace the samples back to me ☐
9. I understand that my samples may be used in the future for more advanced tests as there are more scientific advances. ☐
10. I understand that the samples may be sent outside Europe and that future tests on the samples will be confined to research on CDI-related conditions. The samples will only be identified by a code. ☐
11. I agree to take part in the study ☐

.....	.....	.....
Name of patient	Date	Signature
.....	.....	.....
Name of person	Date	Signature

1 copy for the researcher, 1 copy for the patient, 1 copy for the notes

2: Case Report Form (CRF) of individual susceptibility to *Clostridium difficile* infection (CDI) study

Study Code | Site Code: | Subject Number:

UNIVERSITY OF LIVERPOOL

Clostridium Difficile Toxin Disease

Case Report Form

Study Code:	
Site Code:	
Subject Number:	
Patient Recruitment Date:	DD/MMM/YYYY
Patient Initials:	
Additional Research Number	

Only complete this box if the patient has been allocated a Study ID number and then subsequently changed groups (not withdrawn from study previously)

Recruiting Health Professional;

Name:	
Hospital/GP Practice:	
Work Telephone Number:	
Work Email Address:	

Chief Investigator: Prof M. Pirmohamed
The Wolfson Centre for Personalised Medicine
Department of Pharmacology
University of Liverpool
Block A: Waterhouse Buildings
1-5 Brownlow Street
Liverpool, L69 3GL

Study Code | Site Code: | Subject Number:

General Guidelines for CRF Completion

Please complete the Case Report Form (CRF) as thoroughly as possible and then post, fax a photocopy or scan and email a copy of the completed CRF to the lead coordinating centre.

The structure of the CRF is shown in the following diagram;

Even Pages	**Odd Pages**
Contain notes on how to complete the adjacent ➡ odd numbered page	To be completed

All forms should be completed in black ink in a clear manner. Any changes or corrections should be made by drawing a line through the data, entering the corrected information and initialling and dating the change.

Following standard notation should be used in the event that values or answers cannot be provided:

- NA: Not applicable
- NK: Not known
- ND: Not done
- NR: Not retrievable/Not available

[1] Control Group 1

Exclusions

- Recent laxative use / enema
- Recent bowel surgery
- Overflow diarrhoea
- Enteral feeding
- An acute flare up of inflammatory bowel disease

[2] Control Group 2

Exclusions

- Acute disease
- Infectious disease
- Enteric disease
- Inflammatory bowel disease
- Diarrhoea (acute or chronic)

Study Code | Site Code: | Subject Number:

Identification of Patient Group

A. Has the subject developed diarrhoea which has been confirmed by laboratory diagnosis of CDI?	Yes ☐ *Patient should be considered for "Case Group"*	No ☐
B. Has the subject developed diarrhoea and either I. Had a negative CDI result [1] Or II. Positive GDH result (exclusions do not apply to this cohort	Yes ☐ *Patient should be considered for "Control Group 1"*	No ☐
C. Has the subject shown no history of CDI in the past 12 months and been in generally good health? [2]	Yes ☐ *Participant should be considered for "Control Group 2"*	No ☐
D. Has the subject developed an acute flare up of Inflammatory Bowel Disease with either active Crohn's Disease, Ulcerative Colitis or indeterminant colitis?	Yes ☐ *Patient should be considered for "Control Group 3"*	No ☐

Study Code | Site Code: | Subject Number:

Inclusion/Exclusion criteria – Notes

[1] The patient **must** be given a Patient Information Leaflet and Consent Form to be included in the study. If patient lacks capacity to consent then a personal consultee or a nominated consultee will be approached

[2] If the patient is participating in another study, it is essential to discuss eligibility to participate with the Principal Investigator prior to ticking "yes" or "no".

Study Code | Site Code: | Subject Number:

Inclusion/Exclusion Criteria

Please tick 'yes' or 'no' to all questions

A		Yes	No
1.	Patient willing to take part in study		
2.	Is the patient aged 18 or over?		
3.	Has the study been discussed in full to include the risks, benefits and rights to withdraw? Date Discussed [DD / MMM / YYYY]		
4.	Patient information leaflet read by Patient/Personal Consultee/Nominated Consultee? [1] Version [___] Version dated [DD / MMM / YYYY] Date given [DD / MMM / YYYY]		
5.	Written informed consent obtained? [1] Version [___] Version dated [DD / MMM / YYYY] Date given [DD / MM M/ YYYY]		

Exclusion Criteria

Please tick 'yes' or 'no' to all questions

B		Yes	No
6.	Patient is unwilling to take part		
7.	Patient unable to give informed consent		
8.	Unable to nominate a consultee for patient who lacks capacity		
9.	Patient is, in the opinion of the Investigator, not suitable to participate in the study.		
10.	Patient is taking part in another study and after consultation with Lead Investigator confirmed as 'not eligible'		

Patient Eligible for study [2]	Yes ☐ Patient included in the study Please complete CRF	No ☐ Patient Not included in the study

Study Code | Site Code: | Subject Number:

Recruitment Information – Notes

[1] Please enter **both** the patients date of birth and age at the time of recruitment.

[2] Ethnic origin as self-reported, by the patient or documented in casenotes.

Please use codes as listed:

1. White
2. White Irish
3. Other White
4. Mixed: White and Black Caribbean
5. Mixed: White and Black African
6. Mixed: White and Asian
7. Other mixed background
8. Indian
9. Pakistani
10. Bangladeshi
11. Other Asian background
12. Caribbean
13. African
14. Other Black background
15. Chinese
16. Other ethnic group (please specify)

[3] **Where was the subject recruited**:

A. Hospital Inpatient
B. Hospital Outpatient
C. Other

PLEASE NOTE:
Give as much detail, i.e. ward & room, where applicable for the location of where the subject was recruited from.

[4] **Reason for Admission**:

A. CDI (confirmed)
B. Diarrhoea
C. Other

Study Code | Site Code: | Subject Number:

Recruitment Information

Patient Demographics

Sex	Male ☐ Female ☐
Date of Birth [1]	DD/MMM/YYYY — Age (in years) ☐
Height	. m (2 decimal places)
Weight	. kg (2 decimal places)

Ethnic Origin

Own [2]	☐ Specify ☐ Country of Birth ☐

Recruitment Information

1. Where has the subject been recruited from? [3]	A ☐ B ☐ C ☐ *If an inpatient please complete question 2* *Specify* Please give ward, room / bay / bed space
2. Admission Date	DD/MMM/YYYY N/A ☐
Reason for Admission [4]	A ☐ B ☐ C ☐
If 'Other' (C)	
3. Primary Diagnosis	Specify

Study Code | Site Code: | Subject Number:

Previous And Current Medical History - Notes

[1] Smoking Status

Please categorise as:-

- Current smoker - within past 3 months
- Previous smoker - stopped smoking for more than 3 months
- Non Smoker – Never smoked

[2] Alcohol Intake per week.

Encourage the patient to give the most accurate numerical reading as possible.

Study Code ☐ Site Code: ☐ Subject Number: ☐

Previous And Current Medical History

1. **Diabetes**	Yes ☐ If yes, specify type below No ☐ Type 1 ☐ Type 2 (diet) ☐ Type 2 (tablets) ☐ Type 2 (Insulin) ☐
2. **Respiratory Disease**	Yes ☐ No ☐ Specify ☐
3. **Cardiac Disease**	Yes ☐ No ☐ Specify ☐
4. **Neurological Disease**	Yes ☐ No ☐ Specify ☐
5. **Hepatic Disease**	Yes ☐ No ☐ Specify ☐
6. **Gastrointestinal Disease**	Yes ☐ No ☐ Specify ☐
7. **Urological Conditions**	Yes ☐ No ☐ Specify ☐
8. **Musculoskeletal**	Yes ☐ No ☐ Specify ☐
9. **Other Disease**	Yes ☐ No ☐ Specify ☐ Specify ☐ Specify ☐ Specify ☐ Specify ☐ Specify ☐ Specify ☐ Specify ☐ Specify ☐
10. **Smoking status [1]**	Current ☐ Previous ☐ Never Smoked ☐
11. **Alcohol Intake per week (units)**	< 1 ☐ 1 - 5 ☐ 6 -14 ☐ 15 - 21 ☐ 22 - 49 ☐ > 50 ☐

Study Code | Site Code: | Subject Number:

Past Medical History of Allergic Disease - Notes

[1] **Mobility:**
- **A.** I have no problems in walking about
- **B.** I have some problems in walking about
- **C.** I am confined to bed

[2] **Self-Care:**
- **A.** I have no problems with self care
- **B.** I have some problems washing and drying myself
- **C.** I am unable to wash or dress myself

[3] **Usual Activities** (e.g. work, study, housework, family or leisure activates):
- **A.** I have no problems performing my usual activities
- **B.** I have some problems performing my usual activities
- **C.** I am unable to perform my usual activities

[4] **Pain/Discomfort:**
- **A.** I have no pain or discomfort
- **B.** I have moderate pain or discomfort
- **C.** I have extreme pain or discomfort

[5] **Anxiety/Depression:**
- **A.** I am not anxious or depressed
- **B.** I am moderately anxious or depressed
- **C.** I am extremely anxious or depressed

[6] **Best imaginable health state:**
Please indicate **with a vertical line** on the scale, where 0 is the worst and 100 is the best, how you would describe your health state **today**. For example:

Study Code | Site Code: | Subject Number:

Past Medical History of Allergic Disease

Does the patient have any known history of allergic disease?	Asthma Yes ☐ No ☐ NK ☐ Eczema Yes ☐ No ☐ NK ☐ Hayfever Yes ☐ No ☐ NK ☐ Anaphylaxis Yes ☐ Specify ☐ No ☐ NK ☐ Other Yes ☐ Specify ☐ No ☐ NK ☐

Economics

Patient's Occupation	☐
Hours Per Week (HPW) usually worked by patient? *(If applicable)*	☐
Does the patient normally receive aid by a carer prior to hospitalisation?	Yes ☐ No ☐
Informal Care?	Yes ☐ No ☐ Hours/Week ☐
External agency cover	Yes ☐ No ☐ Hours/Week ☐
Roughly how long for?	☐ Weeks ☐ Months
Is this attributed to CDI?	Yes ☐ No ☐ Not Known ☐
Occupation of informal carer	☐
HPW usually worked by informal carer?	☐ Hours/Week

Mobility [1]	A ☐	B ☐	C ☐
Self Care [2]	A ☐	B ☐	C ☐
Usual Activities [3]	A ☐	B ☐	C ☐
Pain/Discomfort [4]	A ☐	B ☐	C ☐
Anxiety/Depression [5]	A ☐	B ☐	C ☐
Health State [6]	Please indicate on this scale how good or bad your own health is today.		

0 - Worst *100 - Best*

Study Code | Site Code: | Subject Number:

Current Episode Information - Notes

[1] **Date of Onset**:
Please provide date of onset of diarrhoea for this episode or best estimated date.

[2] **Total Number of Stools**:
For patients who have not been tested for CDI from within 48hrs prior to recruitment.

[3] **Clinical Stool Sample**:
Please record date of clinical stool sample collected that alerted Research Nurse to subject.

[4] **Specimen Category:**
Please record the highest Bristol Stool Score from 48 hours prior to being tested for CDI or for patients who have not been tested for CDI from 48 hrs. prior to recruitment.

[5] **Tick all that apply**

- NG – Nasogastric
- PEG – Percutaneous Endoscopic Gastronomy
- TPN – Total Parental Nutrition

[6] Please record usual weight from up to 6 months using most reliable source i.e. case notes, Patient, relatives, GP etc.

[7] Please calculate recent weight loss using usual weight minus current weigh

[8] In last 6 months

Study Code | Site Code: | Subject Number:

Current Episode Information

Date of Onset [1]	DD/MMM/YYYY
Total number of stools on worst day	** From 48 hours prior to being tested for CDI [2]
Date of worst day	DD/MMM/YYYY
Clinical Stool sample collected? [3]	Yes ☐ Date & Time collected DD/MMM/YYYY HH:MM NA ☐ No ☐ Reason not collected
Specimen Category based upon Bristol Stool Tool? [4]	5 ☐ 6 ☐ 7 ☐ Other No. ☐ Date DD/MMM/YYYY

Nutritional Information

Type of Diet [5]	Oral ☐ NG / PEG ☐ TPN ☐
Usual weight [6]	. (KG) (2 decimal places) Date DD/MMM/YYYY Source
Unintentional weight-loss in last 3-6 months? [7]	<5% ☐ 5-10% ☐ >10% ☐
Has patient been referred to a dietician? [8]	Yes ☐ No ☐
If 'Yes', Date	DD/MMM/YYYY

Study Code | Site Code: | Subject Number:

Blood Sample Collections - Notes

[1] You will be asked to collect a blood sample by the lead co-ordinating centre. A total of 32ml to be collected in vacuette containers, 1 urine sample and 4 swab/gauzes for collection of spores (gauzes and media provided)

[2] **CRP Sample**:
Please record CRP result from date of clinical stool sample collection up to date of recruitment or if no clinical sample collected from 48 hours prior up until recruitment date. If not collected as part of clinical care please obtain clinical sample

[3] **Skin Swabs**
A. Hands & Arms
B. Neck, Chest & Abdomen

[4] **Environmental Swabs:**
- Please collect for all study arms if an in-patients
- For outpatients / community patients do not collect environmental swabs

Study Code | Site Code: | Subject Number:

Blood Sample Collections [1]

Blood sample collected for DNA?	Yes ☐ No ☐ N/A ☐	Date & time collected: DD/MM/YYYY – HH:MM Reason not collected:
Blood sample collected for Plasma?	Yes ☐ No ☐ N/A ☐	Date & time collected: DD/MM/YYYY – HH:MM Reason not collected:
Blood sample collected for Serum?	Yes ☐ No ☐ N/A ☐	Date & time collected: DD/MM/YYYY – HH:MM Reason not collected:
2 x Transcriptomic samples collected?	Yes ☐ No ☐ N/A ☐	Date & time collected: DD/MM/YYYY – HH:MM Reason not collected:
Blood sample collected for CRP? [2	Yes ☐ No ☐ N/A ☐	Date & time collected: DD/MM/YYYY – HH:MM Reason not collected:
Urine sample collected? *(Universal container)*	Yes ☐ No ☐ N/A ☐	Date & time collected: DD/MM/YYYY – HH:MM Reason not collected:
Skin swabs collected? **Site of swab [3]**	Yes ☐ A ☐ B ☐ No ☐ N/A ☐	Date & time collected: DD/MM/YYYY – HH:MM Reason not collected:
Environmental Swabs [4] from toilet areas sink, support rail, flush handle, top and underside of seat and handles collected?	Yes ☐ No ☐ N/A ☐	Date & time collected: DD/MM/YYYY – HH:MM Reason not collected:
Environmental swabs [4] from handles, bed rails, bedside table, call bell and door handle?	Yes ☐ No ☐ N/A ☐	Date & time collected: DD/MM/YYYY – HH:MM Reason not collected:

Study Code | Site Code: | Subject Number:

Stool Sample Collection - Notes

[1] request a further stool sample from patient upon recruitment

Study Code | Site Code: | Subject Number:

Stool Sample Collection

Stool sample collected? [1]	Yes ☐	Date & time collected: dd/MMM/YYYY – hh:mm
	No ☐	Reason not collected:
	N/A ☐	

Study Code | Site Code: | Subject Number:

Expenses & Case Report Form Sign-Off - Notes

[1] Please note patients travel expenses should **only** be paid for **research visits** i.e. if the research is conducted whilst the patient is an inpatient or attending for a clinical outpatients appointment travel expenses should **not** be paid.

Study Code | Site Code: | Subject Number:

Expenses & Case Report Form Sign-Off [1]

Where was the research visit conducted?	Patients Home ☐ Ward/ Clinic ☐ Care Facility ☐ Other ☐
Has patient been given an expense remittance slip?	Yes ☐ No ☐ **If yes, please file a copy of any receipts and the remittance slip in the CRF**
Travel Expenses *(Please tick all that apply and give amounts)*	Bus / Taxi fare Yes ☐ £ Petrol allowance & Parking Yes ☐ £ Other Yes ☐ £ Total £

Print Name			
Signature (of person completing CRF)		**Date**	DD/MM/YYYY

Please post or fax a photocopy of the completed case report form to the lead site:

Margaret Little & Rachael Hornby
The Wolfson Centre for Personalised Medicine
Department of Molecular and Clinical Pharmacology
Institute of Translational Medicine
University of Liverpool
Block A: Waterhouse Buildings
1-5 Brownlow Street
Liverpool
L69 3GL
Tel: (+44) 151 794 5539
Fax: (+44) 151 794 5059

Email: margaret.little@rlbuht.nhs.uk or rachael.hornby@rlbuht.nhs.uk

3: Multivariable analysis of PCT (log_{10}) tested within 2 days after toxin test as covariate and CDI diagnosis as outcome

	variable	Odds ratio	95% CI	p
<2	PCT (log_{10})	1.63	1.04-2.58	0.035
	age	1.03	1.00-1.06	0.065

4: Multivariable analysis of PCT (log_{10}) tested within 3 days after toxin test as covariate and CDI diagnosis as outcome

	variable	Odds ratio	95% CI	p
<3	PCT (log_{10})	1.19	0.98-1.44	0.075
	age	1.03	1.01-1.05	<0.001
	WCC	1.03	1.00-1.07	0.050

5: Multivariable analysis of PCT (log_{10}) tested within 4 days after toxin test as covariate and CDI diagnosis as outcome

	variable	Odds ratio	95% CI	p
	PCT (log_{10})	1.13	0.97-1.32	0.110
<4	age	1.02	1.01-1.03	0.003
	CCI	1.18	1.01-1.37	0.033
	WCC	1.05	1.01-1.08	0.005

6: Multivariable analysis of PCT (log_{10}) tested within 5 days after toxin test as covariates and CDI diagnosis as outcome

	variable	Odds ratio	95% CI	p
	PCT (log_{10})	1.12	0.98-1.29	0.089
<5	age	1.02	1.01-1.03	0.004
	pre-test	1.01	1.00-1.03	0.047
	CCI	1.21	1.06-1.39	0.006
	WCC	1.06	1.02-1.09	0.001

7: Multivariable analysis of PCT (log_{10}) tested in all time points after toxin test as covariates and CDI diagnosis as outcome

	variable	Odds ratio	95% CI	p
	PCT (log_{10})	1.12	0.99-1.27	0.075
all time points	age	1.02	1.01-1.03	0.002
	pre-test	1.02	1.01-1.03	0.001
	CCI	1.19	1.05-1.35	0.006
	WCC	1.06	1.03-1.08	<0.001

8: Multivariable analysis of CRP (log_{10}) tested on the same day or before toxin test as covariate and CDI diagnosis as outcome

	variable	Odds ratio	95% CI	p
<0	CRP (log_{10})	1.16	0.89-1.53	0.271
	pre-test	1.03	1.01-1.06	0.010

9: Multivariable analysis of CRP (log_{10}) tested before and within 1 day after toxin test as covariate and CDI diagnosis as outcome

	variable	Odds ratio	95% CI	p
<1	CRP (log_{10})	1.13	0.91-1.39	0.277
	pre-test	1.03	1.01-1.05	0.003
	WCC	1.06	1.02-1.10	0.004

10: Multivariable analysis of CRP (log_{10}) tested before and within 2 days after toxin test as covariate and CDI diagnosis as outcome

	variable	Odds ratio	95% CI	p
<2	CRP (log_{10})	1.18	0.98-1.42	0.072
	pre-test	1.02	1.01-1.03	0.005
	WCC	1.06	1.03-1.10	0.001

11: Multivariable analysis of CRP (log_{10}) tested before and within 3 days after toxin test as covariate and CDI diagnosis as outcome

	variable	Odds ratio	95% CI	p
<3	CRP (log_{10})	1.29	1.08-1.53	0.005
	pre-test	1.02	1.01-1.03	0.001
	WCC	1.05	1.01-1.08	0.005
	CCI	1.16	1.01-1.33	0.041

12: Multivariable analysis of CRP (log_{10}) tested before and within 4 days after toxin test as covariate and CDI diagnosis as outcome

	variable	Odds ratio	95% CI	p
<4	CRP (log_{10})	1.32	1.12-1.56	0.001
	pre-test	1.02	1.01-1.03	0.003
	WCC	1.04	1.01-1.07	0.008

13: Multivariable analysis of CRP (log_{10}) tested before and within 5 days after toxin test as covariate and CDI diagnosis as outcome

	variable	Odds ratio	95% CI	p
<5	CRP (log_{10})	1.33	1.13-1.56	0.001
	pre-test	1.02	1.01-1.03	0.002
	WCC	1.04	1.01-1.07	0.004

14: Multivariable analysis of CRP (log_{10}) tested before and within 6 days after toxin test as covariate and CDI diagnosis as outcome

	variable	Odds ratio	95% CI	p
<6	CRP (log_{10})	1.35	1.14-1.58	<0.001
	pre-test	1.02	1.01-1.03	0.002
	WCC	1.04	1.01-1.07	0.004

15: Multivariable analysis of CRP (log_{10}) tested before and within 7 days after toxin test as covariate and CDI diagnosis as outcome

	variable	Odds ratio	95% CI	p
<7	CRP (log_{10})	1.35	1.15-1.59	<0.001
	pre-test	1.02	1.01-1.03	0.002
	WCC	1.04	1.01-1.07	0.005

16: Multivariable analysis of CRP (log_{10}) tested before and within 8 days after toxin test as covariate and CDI diagnosis as outcome

	variable	Odds ratio	95% CI	p
<8	CRP (log_{10})	1.34	1.14-1.58	<0.001
	pre-test	1.02	1.01-1.03	0.002
	WCC	1.04	1.02-1.08	0.003

17: Multivariable analysis of CRP (log_{10}) tested in all time points as covariate and CDI diagnosis as outcome

	variable	Odds ratio	95% CI	p
all time points	CRP (log_{10})	1.35	1.16-1.57	<0.001
	pre-test	1.02	1.01-1.03	0.001
	WCC	1.04	1.01-1.07	0.004
	age	1.02	1.01-1.03	0.002
	CCI	1.18	1.04-1.34	0.010

18: Cox proportional hazards of multivariable analysis for time to discharge and PCT (log10) tested within 2 days after toxin test

	variable	Haz ratio	95% CI	p
<2	PCT (log_{10})	1.07	0.88-1.31	0.480
	mode of acquisition	0.20	0.11-0.37	<0.001

19: Cox proportional hazards of multivariable analysis for time to discharge and PCT (log10) tested within 3 days after toxin test

	variable	Haz ratio	95% CI	p
<3	PCT (log_{10})	0.87	0.80-0.95	0.002
	mode of acquisition	0.33	0.25-0.44	<0.001
	age	0.99	0.98-1.00	0.001

20: Cox proportional hazards of multivariable analysis for time to discharge and PCT (log10) tested within 4 days after toxin test

	variable	Haz ratio	95% CI	p
<4	PCT (log_{10})	0.93	0.87-1.00	0.061
	mode of acquisition	0.33	0.26-0.42	<0.001
	age	0.99	0.98-1.00	0.003
	toxin	0.72	0.57-0.90	0.004

21: Cox proportional hazards of multivariable analysis for time to discharge and PCT (log10) tested within 5 days after toxin test

	variable	Haz ratio	95% CI	p
<5	PCT (log_{10})	0.93	0.87-0.99	0.028
	mode of acquisition	0.35	0.29-0.44	<0.001
	age	0.99	0.99-1.00	0.035
	toxin	0.71	0.58-0.87	0.001
	albumin	1.01	1.00-1.02	0.031

22: Cox proportional hazards of multivariable analysis for time to discharge and PCT (log10) tested in all time points after toxin test

	variable	Haz ratio	95% CI	p
all time points	PCT (log_{10})	0.93	0.88-0.99	0.029
	mode of acquisition	0.32	0.26-0.39	<0.001
	toxin	0.65	0.54-0.77	<0.001
	albumin	1.01	1.00-1.02	0.003

23: Cox proportional hazards of multivariable analysis for time to discharge and CRP (log10) tested on the same day or before toxin test

	variable	Haz ratio	95% CI	p
<0	CRP (log_{10})	0.93	0.81-1.07	0.307
	toxin	0.60	0.44-0.84	0.002
	mode of acquisition	0.33	0.23-0.48	<0.001

24: Cox proportional hazards of multivariable analysis for time to discharge and CRP (log10) tested before and within 1 day after toxin test

	variable	Haz ratio	95% CI	p
<1	CRP (log_{10})	0.98	0.89-1.09	0.746
	toxin	0.65	0.51-0.84	0.001
	mode of acquisition	0.34	0.26-0.44	<0.001
	albumin	1.01	1.00-1.02	0.040

25: Cox proportional hazards of multivariable analysis for time to discharge and CRP (log10) tested before and within 2 days after toxin test

	variable	Haz ratio	95% CI	p
<2	CRP (log_{10})	0.98	0.90-1.07	0.627
	toxin	0.67	0.54-0.84	0.001
	mode of acquisition	0.32	0.25-0.40	<0.001
	albumin	1.01	1.00-1.02	0.007

26: Cox proportional hazards of multivariable analysis for time to discharge and CRP (log10) tested before and within 3 days after toxin test

	variable	Haz ratio	95% CI	p
<3	CRP (log_{10})	0.94	0.87-1.02	0.152
	toxin	0.67	0.54-0.83	<0.001
	mode of acquisition	0.29	0.24-0.36	<0.001

27: Cox proportional hazards of multivariable analysis for time to discharge and CRP (log10) tested before and within 4 days after toxin test

	variable	Haz ratio	95% CI	p
<4	CRP (log_{10})	0.96	0.88-1.04	0.275
	toxin	0.66	0.53-0.80	<0.001
	mode of acquisition	0.31	0.25-0.38	<0.001
	albumin	1.01	1.00-1.02	0.013

28: Cox proportional hazards of multivariable analysis for time to discharge and CRP (log10) tested before and within 5 days after toxin test

	variable	Haz ratio	95% CI	p
<5	CRP (log_{10})	0.93	0.86-1.01	0.071
	toxin	0.64	0.53-0.78	<0.001
	mode of acquisition	0.32	0.26-0.40	<0.001
	albumin	1.01	1.00-1.02	0.017

29: Cox proportional hazards of multivariable analysis for time to discharge and CRP (log10) tested before and within 6 days after toxin test

	variable	Haz ratio	95% CI	p
<6	CRP (log_{10})	0.93	0.86-1.01	0.096
	toxin	0.65	0.54-0.79	<0.001
	mode of acquisition	0.33	0.27-0.40	<0.001
	albumin	1.01	1.00-1.02	0.018

30: Cox proportional hazards of multivariable analysis for time to discharge and CRP (log10) tested before and within 7 days after toxin test

	variable	Haz ratio	95% CI	p
<7	CRP (log_{10})	0.94	0.86-1.01	0.100
	toxin	0.65	0.53-0.79	<0.001
	mode of acquisition	0.33	0.27-0.40	<0.001
	albumin	1.01	1.00-1.02	0.021

31: Cox proportional hazards of multivariable analysis for time to discharge and CRP (log10) tested before and within 8 days after toxin test

	variable	Haz ratio	95% CI	p
<8	CRP (log_{10})	0.94	0.86-1.01	0.098
	toxin	0.65	0.53-0.79	<0.001
	mode of acquisition	0.33	0.27-0.40	<0.001
	albumin	1.01	1.00-1.02	0.021

32: Cox proportional hazards of multivariable analysis for time to discharge and CRP (log10) tested in all time points

	variable	Haz ratio	95% CI	p
all time points	CRP (log_{10})	0.94	0.86-1.01	0.098
	toxin	0.65	0.53-0.79	<0.001
	mode of acquisition	0.33	0.27-0.40	<0.001
	albumin	1.01	1.00-1.02	0.021

33: Multivariable analysis of PCT (log_{10}) tested within 2 days after toxin test as covariate and short-term mortality as outcome

	variable	Odds ratio	95% CI	p
<2	PCT (log_{10})	1.69	0.64-4.48	0.291
	CCI	2.09	1.08-4.05	0.029

34: Multivariable analysis of PCT (log_{10}) tested within 3 days after toxin test as covariate and short-term mortality as outcome

	variable	Odds ratio	95% CI	p
<3	PCT (log_{10})	1.32	0.96-1.81	0.086
	CCI	1.34	1.00-1.80	0.050

35: Multivariable analysis of PCT (log_{10}) tested within 4 days after toxin test as covariate and short-term mortality as outcome

	variable	Odds ratio	95% CI	p
<4	PCT (log_{10})	1.33	1.00-1.76	0.048

36: Multivariable analysis of PCT (log_{10}) tested within 5 days after toxin test as covariate and short-term mortality as outcome

	variable	Odds ratio	95% CI	p
<5	PCT (log_{10})	1.30	1.02-1.66	0.033
	CCI	1.35	1.08-1.68	0.008

37: Multivariable analysis of PCT (log_{10}) tested in all time points after toxin test as covariate and short-term mortality as outcome

	variable	Odds ratio	95% CI	p
all time points	PCT (log_{10})	1.28	1.01-1.62	0.038
	CCI	1.06	1.62-2.54	0.011
	mode of acquisition	1.03	7.78-2.02	0.044

38: Multivariable analysis of CRP (log_{10}) tested on the same day of before toxin test as covariate and short-term mortality as outcome

	variable	Odds ratio	95% CI	p
<0	CRP (log_{10})	3.03	1.09-8.44	0.034

39: Multivariable analysis of CRP (log_{10}) tested before and within 1 day after toxin test as covariate and short-term mortality as outcome

	variable	Odds ratio	95% CI	p
<1	CRP (log_{10})	1.85	0.95-3.62	0.073

40: Multivariable analysis of CRP (log_{10}) tested before and within 2 days after toxin test as covariate and short-term mortality as outcome

	variable	Odds ratio	95% CI	p
<2	CRP (log_{10})	1.66	0.91-3.02	0.097
	mode of acquisition	8.02	1.03-62.64	0.047

41: Multivariable analysis of CRP (log_{10}) tested before and within 3 days after toxin test as covariate and short-term mortality as outcome

	variable	Odds ratio	95% CI	p
<3	CRP (log_{10})	1.42	0.90-2.24	0.128
	mode of acquisition	5.22	1.18-23.06	0.029

42: Multivariable analysis of CRP (log_{10}) tested before and within 4 days after toxin test as covariate and short-term mortality as outcome

	variable	Odds ratio	95% CI	p
<4	CRP (log_{10})	1.40	0.92-2.12	0.116
	CCI	1.27	1.02-1.58	0.036
	mode of acquisition	3.64	1.05-12.66	0.042

43: Multivariable analysis of CRP (log_{10}) tested before and within 5 days after toxin test as covariate and short-term mortality as outcome

	variable	Odds ratio	95% CI	p
<5	CRP (log_{10})	1.35	0.91-2.01	0.136
	CCI	1.27	1.02-1.58	0.031
	mode of acquisition	3.90	1.13-13.49	0.031

44: Multivariable analysis of CRP (log_{10}) tested before and within 6 days after toxin test as covariate and short-term mortality as outcome

	variable	Odds ratio	95% CI	p
<6	CRP (log_{10})	1.35	0.91-2.01	0.137
	CCI	1.27	1.02-1.58	0.031
	mode of acquisition	3.98	1.15-13.76	0.029

45: Multivariable analysis of CRP (log_{10}) tested before and within 7 days after toxin test as covariate and short-term mortality as outcome

	variable	Odds ratio	95% CI	p
<7	CRP (log_{10})	1.35	0.91-2.00	0.139
	CCI	1.27	1.02-1.58	0.031
	mode of acquisition	3.97	1.15-13.70	0.029

46: Multivariable analysis of CRP (log_{10}) tested before and within 8 days after toxin test as covariate and short-term mortality as outcome

	variable	Odds ratio	95% CI	p
<8	CRP (log_{10})	1.35	0.91-2.01	0.136
	CCI	1.27	1.02-1.58	0.031
	mode of acquisition	3.95	1.14-13.66	0.030

47: Multivariable analysis of CRP (log_{10}) tested in all time points as covariate and short-term mortality as outcome

	variable	Odds ratio	95% CI	p
all time points	CRP (log_{10})	1.42	0.96-2.10	0.079
	CCI	1.25	1.01-1.55	0.039
	mode of acquisition	3.39	1.13-10.19	0.030

48: Multivariable analysis of PCT (log_{10}) tested within 2 days after toxin test as covariate and long-term mortality as outcome

	variable	Odds ratio	95% CI	p
<2	PCT (log_{10})	1.40	0.85-2.29	0.188
	CCI	1.80	1.14-2.83	0.011

49: Multivariable analysis of PCT (log_{10}) tested within 3 days after toxin test as covariate and long-term mortality as outcome

	variable	Odds ratio	95% CI	p
<3	PCT (log_{10})	1.43	1.15-1.77	0.001
	CCI	1.52	1.22-1.89	<0.001
	albumin	0.97	0.95-1.00	0.025

50: Multivariable analysis of PCT (log_{10}) tested within 4 days after toxin test as covariate and long-term mortality as outcome

	variable	Odds ratio	95% CI	p
<4	PCT (log_{10})	1.33	1.12-1.58	0.001
	CCI	1.36	1.15-1.60	<0.001
	age	1.02	1.00-1.04	0.045
	mode of acquisition	2.07	1.16-3.71	0.014

51: Multivariable analysis of PCT (log_{10}) tested within 5 days after toxin test as covariate and long-term mortality as outcome

	variable	Odds ratio	95% CI	p
<5	PCT (log_{10})	1.28	1.10-1.48	0.002
	CCI	1.45	1.25-1.68	<0.001
	age	1.02	1.01-1.04	0.008
	mode of acquisition	2.15	1.29-3.59	0.003

52: Multivariable analysis of PCT (log_{10}) tested in all time points after toxin test as covariate and long-term mortality as outcome

	variable	Odds ratio	95% CI	p
all time points	PCT (log_{10})	1.22	1.06-1.40	0.005
	CCI	1.42	1.24-1.61	<0.001
	age	1.03	1.01-1.04	0.001
	mode of acquisition	1.58	1.02-2.44	0.041

53: Multivariable analysis of CRP (log_{10}) tested on the same day or before toxin test as covariate and long-term mortality as outcome

	variable	Odds ratio	95% CI	p
<0	CRP (log_{10})	1.32	0.92-1.90	0.136
	age	1.04	1.01-1.07	0.010
	CCI	1.42	1.09-1.84	0.009

54: Multivariable analysis of CRP (log_{10}) tested before and within 1 day after toxin test as covariate and long-term mortality as outcome

	variable	Odds ratio	95% CI	p
<1	CRP (log_{10})	1.33	1.01-1.75	0.040
	age	1.04	1.01-1.06	0.002
	CCI	1.40	1.16-1.71	0.001

55: Multivariable analysis of CRP (log_{10}) tested before and within 2 days after toxin test as covariate and long-term mortality as outcome

	variable	Odds ratio	95% CI	p
<2	CRP (log_{10})	1.41	1.11-1.80	0.005
	age	1.03	1.01-1.05	0.007
	CCI	1.50	1.26-1.79	<0.001

56: Multivariable analysis of CRP (log_{10}) tested before and within 3 days after toxin test as covariate and long-term mortality as outcome

	variable	Odds ratio	95% CI	p
<3	CRP (log_{10})	1.27	1.03-1.56	0.026
	age	1.02	1.01-1.04	0.007
	CCI	1.40	1.21-1.63	<0.001
	mode of acquisition	1.89	1.11-3.21	0.018

57: Multivariable analysis of CRP (log_{10}) tested before and within 4 days after toxin test as covariate and long-term mortality as outcome

	variable	Odds ratio	95% CI	p
	CRP (log_{10})	1.22	1.00-1.48	0.055
<4	age	1.02	1.01-1.04	0.007
	CCI	1.44	1.25-1.66	<0.001
	mode of acquisition	1.68	1.02-2.78	0.042

58: Multivariable analysis of CRP (log_{10}) tested before and within 5 days after toxin test as covariate and long-term mortality as outcome

	variable	Odds ratio	95% CI	p
	CRP (log_{10})	1.15	0.95-1.39	0.141
<5	age	1.02	1.00-1.03	0.017
	CCI	1.43	1.24-1.65	<0.001
	mode of acquisition	1.66	1.03-2.68	0.038

59: Multivariable analysis of CRP (log_{10}) tested before and within 6 days after toxin test as covariate and long-term mortality as outcome

	variable	Odds ratio	95% CI	p
	CRP (log_{10})	1.17	0.97-1.41	0.094
<6	age	1.02	1.00-1.03	0.016
	CCI	1.44	1.25-1.65	<0.001
	mode of acquisition	1.61	1.01-2.58	0.047

60: Multivariable analysis of CRP (log_{10}) tested before and within 7 days after toxin test as covariate and long-term mortality as outcome

	variable	Odds ratio	95% CI	p
	CRP (log_{10})	1.17	0.97-1.41	0.097
<7	age	1.02	1.00-1.03	0.016
	CCI	1.44	1.25-1.65	<0.001
	mode of acquisition	1.60	1.00-2.57	0.049

61: Multivariable analysis of CRP (log_{10}) tested before and within 8 days after toxin test as covariate and long-term mortality as outcome

	variable	Odds ratio	95% CI	p
<8	CRP (log_{10})	1.16	0.97-1.40	0.110
	age	1.02	1.00-1.03	0.017
	CCI	1.44	1.25-1.66	<0.001
	mode of acquisition	1.63	1.02-2.61	0.042

62: Multivariable analysis of CRP (log_{10}) tested in all time points as covariate and long-term mortality as outcome

	variable	Odds ratio	95% CI	p
all time points	CRP (log_{10})	1.18	0.99-1.40	0.066
	age	1.02	1.01-1.04	0.002
	CCI	1.43	1.26-1.64	<0.001

63: Cox proportional hazards of multivariable analysis for time to death within 1 year and PCT (log10) tested within 2 days after toxin test

	variable	Haz ratio	95% CI	p
<2	PCT (log_{10})	1.26	0.82-1.94	0.295

64: Cox proportional hazards of multivariable analysis for time to death within 1 year and PCT (log10) tested within 3 days after toxin test

	variable	Haz ratio	95% CI	p
<3	PCT (log_{10})	1.14	0.98-1.32	0.083
	CCI	1.19	1.04-1.38	0.014

65: Cox proportional hazards of multivariable analysis for time to death within 1 year and PCT (log10) tested within 4 days after toxin test

	variable	Haz ratio	95% CI	p
<4	PCT (log_{10})	1.14	1.00-1.30	0.056
	CCI	1.16	1.02-1.31	0.023

66: Cox proportional hazards of multivariable analysis for time to death within 1 year and PCT (log10) tested within 5 days after toxin test

	variable	Haz ratio	95% CI	p
<5	PCT (log_{10})	1.11	0.99-1.25	0.068
	CCI	1.17	1.06-1.29	0.002
	study phase	1.61	1.03-2.51	0.037

67: Cox proportional hazards of multivariable analysis for time to death within 1 year and PCT (log10) tested in all time points after toxin test

	variable	Haz ratio	95% CI	p
all time points	PCT (log_{10})	1.09	0.98-1.21	0.126
	CCI	1.15	1.05-1.26	0.003
	study phase	1.66	1.14-2.41	0.008

68: Cox proportional hazards of multivariable analysis for time to death within 1 year and CRP (log10) tested on the same day or before toxin test

	variable	Haz ratio	95% CI	p
<0	CRP (log_{10})	1.37	0.98-1.92	0.064

69: Cox proportional hazards of multivariable analysis for time to death within 1 year and CRP (log10) tested before and within 1 day after toxin test

	variable	Haz ratio	95% CI	p
<1	CRP (log_{10})	1.29	1.01-1.64	0.040

70: Cox proportional hazards of multivariable analysis for time to death within 1 year and CRP (log10) tested before and within 2 days after toxin test

	variable	Haz ratio	95% CI	p
<2	CRP (log_{10})	1.34	1.09-1.65	0.005

71: Cox proportional hazards of multivariable analysis for time to death within 1 year and CRP (log10) tested before and within 3 days after toxin test

	variable	Haz ratio	95% CI	p
<3	CRP (log_{10})	1.26	1.05-1.50	0.011
	study phase	1.92	1.22-3.03	0.005
	mode of acquisition	1.87	1.17-3.00	0.009

72: Cox proportional hazards of multivariable analysis for time to death within 1 year and CRP (log10) tested before and within 4 days after toxin test

	variable	Haz ratio	95% CI	p
<4	CRP (log_{10})	1.26	1.06-1.49	0.009
	CCI	1.14	1.04-1.26	0.007

73: Cox proportional hazards of multivariable analysis for time to death within 1 year and CRP (log10) tested before and within 5 days after toxin test

	variable	Haz ratio	95% CI	p
<5	CRP (log_{10})	1.22	1.04-1.44	0.014
	CCI	1.14	1.03-1.26	0.010
	study phase	1.74	1.14-2.65	0.010

74: Cox proportional hazards of multivariable analysis for time to death within 1 year and CRP (log10) tested before and within 6 days after toxin test

	variable	Haz ratio	95% CI	p
<6	CRP (log_{10})	1.23	1.05-1.44	0.011
	CCI	1.14	1.04-1.26	0.007
	study phase	1.77	1.17-2.68	0.007

75: Cox proportional hazards of multivariable analysis for time to death within 1 year and CRP (log10) tested before and within 7 days after toxin test

	variable	Haz ratio	95% CI	p
<7	CRP (log_{10})	1.23	1.05-1.44	0.011
	CCI	1.14	1.04-1.26	0.007
	study phase	1.77	1.17-2.68	0.007

76: Cox proportional hazards of multivariable analysis for time to death within 1 year and CRP (log10) tested before and within 2 days after toxin test

	variable	Haz ratio	95% CI	p
<8	CRP (log_{10})	1.23	1.05-1.44	0.012
	CCI	1.14	1.04-1.26	0.007
	study phase	1.80	1.19-2.71	0.005

77: Cox proportional hazards of multivariable analysis for time to death within 1 year and CRP (log10) tested in all time points

	variable	Haz ratio	95% CI	p
all time points	CRP (log_{10})	1.20	1.03-1.40	0.018
	CCI	1.13	1.03-1.24	0.009
	study phase	1.80	1.21-2.67	0.004

78: Multivariable analysis of PCT (log_{10}) tested within 2 days after toxin test as covariate and CDI severity as outcome

	variable	Odds ratio	95% CI	p
<2	PCT (log_{10})	1.57	0.86-2.87	0.143

79: Multivariable analysis of PCT (log_{10}) tested within 3 days after toxin test as covariate and CDI severity as outcome

	variable	Odds ratio	95% CI	p
<3	PCT (log_{10})	1.56	1.18-2.07	0.002

80: Multivariable analysis of PCT (log_{10}) tested within 4 days after toxin test as covariate and CDI severity as outcome

	variable	Odds ratio	95% CI	p
<4	PCT (log_{10})	1.33	1.09-1.63	0.006

81: Multivariable analysis of PCT (log_{10}) tested within 5 days after toxin test as covariate and CDI severity as outcome

	variable	Odds ratio	95% CI	p
<5	PCT (log_{10})	1.22	1.03-1.45	0.020

82: Multivariable analysis of PCT (log_{10}) tested in all time points after toxin test as covariate and CDI severity as outcome

all time points	variable	Odds ratio	95% CI	p-value
	PCT (log_{10})	1.30	1.11-1.52	0.001

83: Multivariable analysis of CRP (log_{10}) tested on the same day or before toxin test as covariate and CDI severity as outcome

<0	variable	Odds ratio	95% CI	p
	CRP (log_{10})	1.29	0.84-1.98	0.245

84: Multivariable analysis of CRP (log_{10}) tested before or within 1 day after toxin test as covariate and CDI severity as outcome

<1	variable	Odds ratio	95% CI	p
	CRP (log_{10})	1.44	1.07-1.93	0.017

85: Multivariable analysis of CRP (log_{10}) tested before or within 2 days after toxin test as covariate and CDI severity as outcome

<2	variable	Odds ratio	95% CI	p
	CRP (log_{10})	1.53	1.17-1.99	0.002

86: Multivariable analysis of CRP (log_{10}) tested before or within 3 days after toxin test as covariate and CDI severity as outcome

<3	variable	Odds ratio	95% CI	p
	CRP (log_{10})	1.59	1.23-2.05	<0.001

87: Multivariable analysis of CRP (log_{10}) tested before or within 4 days after toxin test as covariate and CDI severity as outcome

<4	variable	Odds ratio	95% CI	p
	CRP (log_{10})	1.54	1.21-1.96	<0.001

88: Multivariable analysis of CRP (log_{10}) tested before or within 5 days after toxin test as covariate and CDI severity as outcome

	variable	Odds ratio	95% CI	p
<5	CRP (log_{10})	1.54	1.22-1.94	<0.001

89: Multivariable analysis of CRP (log_{10}) tested before or within 6 days after toxin test as covariate and CDI severity as outcome

	variable	Odds ratio	95% CI	p
<6	CRP (log_{10})	1.54	1.22-1.94	<0.001

90: Multivariable analysis of CRP (log_{10}) tested before or within 7 days after toxin test as covariate and CDI severity as outcome

	variable	Odds ratio	95% CI	p
<7	CRP (log_{10})	1.54	1.22-1.94	<0.001

91: Multivariable analysis of CRP (log_{10}) tested before or within 8 days after toxin test as covariate and CDI severity as outcome

	variable	Odds ratio	95% CI	p
<8	CRP (log_{10})	1.52	1.21-1.92	<0.001

92: Multivariable analysis of CRP (log_{10}) tested in all time points as covariate and CDI severity as outcome

	variable	Odds ratio	95% CI	p
all time points	CRP (log_{10})	1.40	1.13-1.73	0.002

93: Multivariable analysis of PCT (log_{10}) tested within 2 days after toxin test as covariate and CDI recurrence as outcome

	variable	Odds ratio	95% CI	p
<2	PCT (log_{10})	0.80	0.33-1.94	0.620

94: Multivariable analysis of PCT (log_{10}) tested within 3 days after toxin test as covariate and CDI recurrence as outcome

	variable	Odds ratio	95% CI	p
<3	PCT (log_{10})	0.94	0.55-1.59	0.806
	age	1.09	1.01-1.18	0.033

95: Multivariable analysis of PCT (log_{10}) tested within 4 days after toxin test as covariate and CDI recurrence as outcome

	variable	Odds ratio	95% CI	p
<4	PCT (log_{10})	1.21	0.89-1.66	0.225
	age	1.06	1.01-1.10	0.010

96: Multivariable analysis of PCT (log_{10}) tested within 5 days after toxin test as covariate and CDI recurrence as outcome

	variable	Odds ratio	95% CI	p
<5	PCT (log_{10})	1.06	0.81-1.39	0.647
	age	1.05	1.02-1.09	0.002

97: Multivariable analysis of PCT (log_{10}) tested in all time points after toxin test as covariate and CDI recurrence as outcome

	variable	Odds ratio	95% CI	p
all time points	PCT (log_{10})	1.01	0.81-1.26	0.960
	age	1.05	1.02-1.07	0.001

98: Multivariable analysis of CRP (log_{10}) tested on the same day or before toxin test as covariate and CDI recurrence as outcome

	variable	Odds ratio	95% CI	p
<0	CRP (log_{10})	1.47	0.65-3.32	0.360
	age	1.06	1.00-1.12	0.050

99: Multivariable analysis of CRP (log_{10}) tested before or within 1 day after toxin test as covariate and CDI recurrence as outcome

	variable	Odds ratio	95% CI	p
<1	CRP (log_{10})	1.13	0.73-1.75	0.596
	age	1.04	1.01-1.08	0.017

100: Multivariable analysis of CRP (log_{10}) tested before or within 2 days after toxin test as covariate and CDI recurrence as outcome

	variable	Odds ratio	95% CI	p
<2	CRP (log_{10})	1.20	0.83-1.72	0.333
	age	1.03	1.01-1.06	0.014

101: Multivariable analysis of CRP (log_{10}) tested before or within 3 days after toxin test as covariate and CDI recurrence as outcome

	variable	Odds ratio	95% CI	p
<3	CRP (log_{10})	1.30	0.91-1.86	0.145
	age	1.04	1.01-1.07	0.006

102: Multivariable analysis of CRP (log_{10}) tested before or within 4 days after toxin test as covariate and CDI recurrence as outcome

	variable	Odds ratio	95% CI	p
<4	CRP (log_{10})	1.28	0.91-1.80	0.153
	age	1.04	1.01-1.06	0.007

103: Multivariable analysis of CRP (log_{10}) tested before or within 5 days after toxin test as covariate and CDI recurrence as outcome

	variable	Odds ratio	95% CI	p
<5	CRP (log_{10})	1.22	0.88-1.69	0.229
	age	1.04	1.02-1.07	0.002

104: Multivariable analysis of CRP (log_{10}) tested before or within 6 days after toxin test as covariate and CDI recurrence as outcome

	variable	Odds ratio	95% CI	p
<6	CRP (log_{10})	1.24	0.90-1.72	0.185
	age	1.04	1.02-1.07	0.001

105: Multivariable analysis of CRP (log_{10}) tested before or within 7 days after toxin test as covariate and CDI recurrence as outcome

	variable	Odds ratio	95% CI	p
<7	CRP (log_{10})	1.24	0.90-1.72	0.185
	age	1.04	1.02-1.07	0.001

106: Multivariable analysis of CRP (log_{10}) tested before or within 8 days after toxin test as covariate and CDI recurrence as outcome

	variable	Odds ratio	95% CI	p
<8	CRP (log_{10})	1.25	0.90-1.73	0.180
	age	1.04	1.02-1.07	0.001

107: Multivariable analysis of CRP (log_{10}) tested in all time points as covariate and CDI recurrence as outcome

	variable	Odds ratio	95% CI	p
all time points	CRP (log_{10})	1.10	0.82-1.48	0.505
	age	1.05	1.02-1.07	0.001

108: Full search strategies

(A) Embase and MEDLINE via Ovid

1 exp *Clostridium difficile*/

2 exp Clostridium Infections/

3 *Clostridium difficile*.mp.

4 Cdifficile.mp.

5 (C adj difficile).mp.

6 difficile clostridium.mp.

7 Cdiff.mp.

8 (C adj diff).mp.

9 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8

10 exp Economics/

11 quality of life/

12 value of life/

13 Quality-adjusted life years/

14 models, economic/

15 markov chains/

16 monte carlo method/

17 decision tree/

18 ec.fs.

19 economic$.tw.

20 (cost or costing or costly or costed).tw.

21 (price or pricing).tw.

22 (pharmacoeconomic or (pharmaco adj economic)).tw. (3242)

23 budget$.tw.

24 expenditure$.tw.

25 (value adj1 (money or monetary)).tw.

26 (fee or fees).tw.

27 "quality of life".tw.

28 qol$.tw.

29 hrqol$.tw.

30 "Quality adjusted life year$".tw.

31 qaly$.tw.

32 cba.tw.

33 cea.tw.

34 cua.tw.

35 utilit$.tw.

36 markov$.tw.

37 monte carlo.tw.

38 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 (1406281)

39 9 and 38

40 humans/ not animals/

41 39 and 40

42 limit 41 to article

(B) EconLit (via EBSCO) and Database of Abstracts of Reviews of Effects, NHS Economic Evaluation database and Health Technology Assessment database (via CRD).

1 *Clostridium difficile*

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