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Original Article

Clostridium difficile carriage in adult cystic fibrosis (CF); implications for patients with CF and the potential for transmission of nosocomial infection

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Abstract

Clostridium difficile is an anaerobic Gram-positive, spore-forming, toxin-producing bacillus transmitted among humans through the faecal–oral route. Despite increasing carriage rates and the presence of *C. difficile* toxin in stool, patients with CF rarely appear to develop typical manifestations of *C. difficile* infection (CDI). In this study, we examined the carriage, toxin production, ribotype distribution and antibiotic susceptibility of *C. difficile* in a cohort of 60 adult patients with CF who were pre-lung transplant. *C. difficile* was detected in 50% (30/60) of patients with CF by culturing for the bacteria. *C. difficile* toxin was detected in 63% (19/30) of *C. difficile*-positive stool samples. All toxin-positive stool samples contained toxigenic *C. difficile* strains harbouring toxin genes, *tcdA* and *tcdB*. Despite the presence of *C. difficile* and its toxin in patient stool, no acute gastrointestinal symptoms were reported. Ribotyping of *C. difficile* strains revealed 16 distinct ribotypes (RT), 11 of which are known to be disease-causing including the hyper-virulent RT078. Additionally, strains RT002, RT014, and RT015, which are common in non-CF nosocomial infection were described. All strains were susceptible to vancomycin, metronidazole, fusidic acid and rifampicin. No correlation was observed between carriage of *C. difficile* or any characteristics of isolated strains and any recorded clinical parameters or treatment received. We demonstrate a high prevalence of hypervirulent, toxigenic strains of *C. difficile* in asymptomatic patients with CF. This highlights the potential role of asymptomatic patients with CF in nosocomial transmission of *C. difficile.*

Keywords: *Clostridium difficile*; Cystic fibrosis; Transmission; Nosocomial infection

1. Introduction

Clostridium difficile is an anaerobic Gram-positive, spore-forming, toxin-producing bacillus transmitted among humans through the faecal–oral route. *C. difficile* colonises the large intestine releasing two protein exotoxins (TcdA and TcdB), which cause colitis in susceptible persons [1,2]. *C. difficile* is the major entero-pathogen of antibiotic-associated diarrhoea (AAD) and is estimated to be responsible for 10–20% of AAD cases including virtually all cases of pseudomembranous colitis [2–4] with an estimated cost of €3 billion per annum among EU states [5] and $1.5 billion per annum in the US [1]. Patients with cystic fibrosis (CF) have been reported to have high rates of carriage of *C. difficile* [6–11] despite rarely manifesting symptoms or developing CDI.

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Cystic fibrosis (CF) is a multisystem disorder due to mutations in the gene which codes for cystic fibrosis conductance regulator (CFTR) protein. CFTR dysfunction in the gut has long been associated with reduced intestinal motility [12], distal intestinal obstruction [13], small intestinal bowel overgrowth [14], and recently altered composition of the gut microbiota [15]. Emerging data highlight the role of the gut and, specifically, the gut microbiota, in influencing gastrointestinal (GI) and non-GI health outcomes for people with CF — the "Lung–Gut Axis" [16–21]. The use of broad-spectrum antibiotics alters the gut microbiota in non-CF populations, and, importantly, encourages the potential overgrowth of *C. difficile* resulting in *C. difficile* infection (CDI) and pseudomembranous colitis [22,23]. With increasing survival there is increasing cumulative antibiotic exposure in CF [24]. Many of the studies exploring *C. difficile* in the CF gut predate this increased antibiotic exposure, though there appears to be a temporal increase in carriage rate with a recent report of up to 47% [6–11,17], compared to 1.1%–15% in healthy adults [25–27]. Studies have also shown variability in the rate of detection of toxin-producing *C. difficile* in the gut of patients with CF [6–11,17]. It is not clear why, while CDI in people with CF pre-transplant is rare [6–8,10,25], patients with CF who are post-transplant have an increased risk of developing, often life-threatening, CDI compared with other immunosuppressed lung transplant recipients [28–30]. There are no published data regarding the association between the carriage of *C. difficile* and any clinical characteristics in CF. Furthermore, there are no data regarding ribotype or antibiotic susceptibility of *C. difficile* strains cultured from patients with CF. To address this, patients attending our CF centre were assessed for carriage of *C. difficile*, presence of its toxin, ribotypes and antibiotic susceptibility with results correlated with CF patient clinical parameters, and a same-site healthy volunteer group.

2. Methods

2.1. Subject recruitment and sample collection

Consecutive adult patients attending the Cork Adult CF centre were recruited prospectively over an 18-month period beginning in January 2012. Patients reporting new gastroenterological symptoms at the time of enrolment were excluded. All participants donated a fresh 5 gramme stool sample at study entry. Post-lung transplant patients with CF were excluded from this analysis. Participant clinical characteristics were recorded at time of study entry. A control group consisting of 99 healthy volunteers aged between 18 and 65 years was recruited from the local university. Exclusion criteria for the control group included coexisting illnesses, gastrointestinal symptoms in the previous 6 months, working in a hospital environment or recent (within previous 6 months) treatment with medications, including antibiotics or probiotics.

2.2. Isolation of C. difficile

Samples were collected from subjects and frozen at −20 °C within 24 h of sampling. *C. difficile* was isolated from stool by ethanol shocking and plating on cycloserine cefoxitin egg yolk agar (Lab M, Bury, United Kingdom), as previously described [31]. Isolates displaying characteristic *C. difficile* morphology and "horse-stable" odour were further assessed microscopically and biochemically. Those found to be Gram-positive anaerobic spore-forming rod-shaped bacteria that were non-haemolytic, L-proline aminopeptidase positive and who gave a positive result using the Oxoid *C. difficile* test kit (Oxoid Basingstoke, UK) were determined to be *C. difficile*. Strains were stocked at −80 °C on Microbank beads (Pro-Lab Diagnostics, Ontario, Canada). For routine use, strains were sub-cultured onto Fastidious Anaerobic Agar (FAA) (Lab M, Heywood, Lancs UK) containing 7% (w/v) defibrinated horse blood and grown anaerobically in a Don Whitley anaerobic chamber at 37 °C.

2.3. Bacterial strains used

C. difficile VPI 10463 (ATCC 43255; A^+/B^+), *C. difficile* CCUG 20309 (A^-/B^+) and *C. difficile* ATCC 43593 (A^-/B^-) were used as positive and negative controls, respectively for the presence of *tcdA* and *tcdB* toxin genes. *C. difficile* ATCC 700057 was used as a control strain for antibiotic susceptibility testing.

2.4. PCR ribotyping

Ribotyping for this study was performed by the *C. difficile* Ribotyping Network for England (CDRNE), at the Microbiology Reference Laboratory, Leeds General Infirmary, United Kingdom. Strains were analysed by capillary gel electrophoresis and compared to over 500 ribotypes housed in the CDRNE ribotype reference library.

2.5. Enzyme immunoassay for C. difficile toxin and detection of toxin genes, tcdA and tcdB by PCR

In vivo toxin production was assessed from stool samples using the commercial kit, Toxin A^+/B^+: ProSpecT II (Oxoid). Assays were performed according to manufacturers' instructions.

For the detection of *tcd*A and *tcd*B genes DNA was extracted from isolates according to Rea et al. [32]. *C. difficile* A^+/B^+, A^-/B^+ and A^-/B^- strains were used as positive and negative controls.

2.6. Antibiotic susceptibility testing

The E-test system (BioMérieux, Hampshire, United Kingdom) was used to screen isolates for antimicrobial resistance against a range of commonly prescribed antibiotics for treating pulmonary exacerbations in patients with CF [33], rifampicin and fusidic acid that have activity against Gram-positive organisms, as well as antibiotics commonly used to treat CDI and those shown to have activity against *C. difficile* including vancomycin and metronidazole. The E-test was performed and interpreted as per the manufacturer's instructions; however Reinforced Clostridial Agar (Merck, Darmstadt, Germany) was substituted. *C. difficile* ATCC 700057 was included as an internal control. Minimum

inhibitory concentration$_{50}$ (MIC_{50}) and MIC_{90}, were calculated as described by Drummond et al. [34]. Strains were deemed susceptible or resistant to the test antibiotic, according to documented pharmacological breakpoint values where available. According to the pharmacological breakpoints recommended by the Swedish Reference Group for Antibiotics (SRGA) (http://www.srga.org), a MIC_{90} of ≤ 2 mg/l indicates susceptibility to both metronidazole and vancomycin.

2.7. Statistical analysis

All statistical analyses were performed using STATA. Chi-square testing was used to examine for a relationship between the carriage of *C. difficile* and gender, genotype, pancreatic sufficiency status and macrolide, PPI and H2-antagonist use. Mann–Whitney testing was used to investigate the relationship between carriage of *C. difficile*, lung function, BMI and number of courses of intravenous antibiotics. In all cases, a 2-sided type I error rate was used with a p-value of < 0.05 accepted as the threshold for statistical significance. Multivariate logistic regression analysis was used to examine the effect of individual clinical variables on the presence or absence of *C. difficile*.

3. Ethical approval

The study was approved by the local research ethics committee (and informed written consent was obtained from all participants in accordance with local research ethics committee guidelines [Clinical Research Ethics Committee of the Cork Teaching Hospitals], Ref: ECM 4 (kk) 01/05/12).

4. Results

60 patients with CF were enrolled along with 99 healthy controls. Table 1 summarises the clinical characteristics of the CF cohort.

C. difficile was isolated from stool samples in 50% (n = 30) of patients with CF and 2% (n = 2) of healthy volunteers. 63% (n = 19) of *C. difficile* culture-positive samples in patients with CF were toxin-positive (Table 2). All *C. difficile* strains isolated from toxin-positive stool samples carried toxin genes, *tcdA* and *tcdB* as revealed by PCR amplification. *C. difficile* strains harbouring toxin genes were not found in any toxin-negative stool samples. During this study period, participants had a higher number of home intravenous antibiotics (IVAB) days (median 28, IQR 0–98) than inpatient IVAB days (median 0, IQR 0–22). However, this did not predict the detection of *C. difficile* in the stool of participants.

PCR ribotyping of the *C. difficile* isolates revealed 16 distinct ribotypes (Table 2).

Eleven toxigenic ribotypes were detected 001, 002, 011, 014, 045, 087, 092, 126, 356, 078 (a known hypervirulent ribotype) and most commonly 046 which, although not commonly associated with CDI in Ireland, has been identified as disease-causing [35]. Four non-toxigenic ribotypes were also revealed, namely 009, 010, 039, and 140. One of the detected ribotypes was a novel ribotype not previously described in the

Table 1
Clinical characteristics of CF study cohort. n = 60.

Age in years (median, [IQR])	27 (24–37)
Male gender	37 (62%)
FEV_1 % predicted (median, [IQR])	65 (46–83)
BMI (median, [IQR])	22.4 (19.4–24.8)
Daily PPI use [a] (n, %)	31 (52%)
Daily macrolide therapy [a]	46 (77%)
Pancreatic insufficiency	49 (82%)
Class 1–3 CFTR mutation	45 (75%)
On CFTR modulator therapy [b]	4 (7%)
Pulmonary exacerbation [b]	14 (23%)
Inpatient [b]	7 (12%)
On intravenous antibiotics for treatment of a P Ex [b]	11 (18%)
On oral antibiotics for treatment of a P Ex [b]	2 (3%)
On maintenance inhaled/nebulised antibiotic [a]	36 (60%)
Breakdown of maintenance inhaled/nebulised antibiotic use [a]	
Colistin n (%)	29 (48)
Tobramycin n (%)	32 (53)
Other n (%)	4 (7)
Not on antibiotic therapy [b]	1 (2%)

IQR = interquartile range, FEV_1 = forced expiratory volume in 1 s, PPI = proton pump inhibitor, BMI = Body Mass Index, P Ex = pulmonary exacerbation.
[a] During previous 12 months.
[b] At time of sample donation.

Table 2
Toxin gene detection, direct stool toxin, ribotype and virulence of *C. difficile* strains detected.

Patient (n = 30)	*tcd-A* [a]	*tcd-B* [b]	Stool toxin (EIA [c])	Ribotype	Virulent
CF3	+	+	+	014	Yes
CF5	+	+	+	002	Yes
CF7	−	−	−	039	No
CF8	+	+	+	126	Yes
CF9	+	+	+	001	Yes
CF10	+	+	+	001	Yes
CF13	−	−	−	140	No
CF15	+	+	+	078	Yes
CF18	−	−	−	140	No
CF21	−	−	−	140	No
CF22	−	−	−	009	No
CF24	−	−	−	010	No
CF26	+	+	+	001	Yes
CF27	+	+	+	046	Yes
CF29	+	+	+	014	Yes
CF34	+	+	+	046	Yes
CF41	+	+	+	045	Yes
CF44	−	−	−	Unknown	No
CF46	−	−	−	039	No
CF47	+	+	+	046	Yes
CF48	+	+	+	046	Yes
CF51	+	+	+	078	Yes
CF52	+	+	+	126	Yes
CF53	−	−	−	140	No
CF61	+	+	+	011	Yes
CF64	+	+	+	092	Yes
CF65	−	−	−	010	No
CF66	−	−	−	140	No
CF71	+	+	+	087	Yes
CF73	+	+	+	356	Yes

[a] *tcd-A* = *C. difficile* toxin A gene.
[b] *tcd-B* = *C. difficile* toxin B gene.
[c] EIA = enzyme immunoassay.

Table 3
Susceptibility of *C. difficile* isolates to commonly used antibiotics.

Antibiotic	Range of sensitivity (mg/l)	Antibiotic break point (mg/l)[a]
Metronidazole	0.016–0.5	2
Vancomycin	0.19–0.5	2
Fusidic acid	0.016–0.75	n/a
Rifampicin	<0.002	n/a
Meropenem	0.125–1	n/a
Linezolid	0.38–4	n/a
Ciprofloxacin	>32	n/a
Ceftazidime	>256	n/a
Tobramycin	>256	n/a
Aztreonam	>256	n/a
Azithromycin	>256	n/a

[a] Antibiotic break point represents the defined EUCAST clinical break point for *C. difficile.*

C. difficile Ribotyping Network database for England and Northern Ireland. Two distinct ribotypes were identified in 2 members of the healthy control group; the rare toxigenic ribotype 062 and a non-toxigenic ribotype 026, neither of which was present in the CF cohort.

The range of activity of an array of antibiotics against the *C. difficile* strains isolated is summarised in Table 3.

All *C. difficile* isolates tested in our study were susceptible to both metronidazole (MIC_{90} of 0.38 μg/ml) and vancomycin (MIC_{90} of 0.75 μg/ml). While there are no published clinical breakpoints for rifampicin, fusidic acid, meropenem or linezolid against *C. difficile*, these drugs performed favourably against the isolates recording MIC_{90} values of <0.002 μg/ml, 0.19 μg/ml, 0.75 μg/ml and 1.5 μg/ml, respectively. High levels of resistance to azithromycin (>256 μg/ml), tobramycin (>256 μg/ml), aztreonam (>256 μg/ml), ceftazidime (>256 μg/ml), and ciprofloxacin (>32 μg/ml) were ubiquitous among isolates.

Chi square testing and Mann–Whitney testing demonstrated no significant associations between the presence of *C. difficile* and PPI use pancreatic insufficiency, maintenance macrolide therapy, intravenous antibiotic use at time of sample donation, use of maintenance inhaled/nebulised antibiotics gender, best FEV_1 % predicted in the previous 12 months, number of days of intravenous antibiotics in the previous 12 months and number of inpatient days in the 3 years prior to study entry (Table 4). There was a trend towards a significant association between the presence of *C. difficile* and older age, higher BMI and the presence of a class 1–3 mutation. Subsequent multivariate logistic regression analysis identified no significant associations between *C. difficile* and recorded clinical variables. 36 of the 60 study participants were taking at least one daily inhaled prophylactic antibiotic (29 using inhaled or nebulised colistin, 32 using inhaled or nebulised tobramycin, 2 using nebulised ceftazidime, 2 using nebulised aztreonam, and 1 using nebulised amikacin) during the study period. 29 of the 36 were alternating between 2 different inhaled antibiotics. 7 were using a single agent, 4 of whom were *C. difficile* positive. 21 of 30 participants who were *C. difficile* positive were taking at least one daily inhaled antibiotic; 17 of the 21 were alternating between 2 inhaled antibiotics with 4 using a single agent. 15 of 30 patients who were *C. difficile* negative were using at least one daily inhaled antibiotic; 12 of the 15 alternated between 2 inhaled antibiotics with 3 using a single agent. There was no association between *C. difficile* carriage and the use of any inhaled antibiotics ($p = 0.140$) or between *C. difficile* carriage and use of colistin ($p = 0.234$) or tobramycin ($p = 0.359$).

5. Discussion

In a CF cohort, we report a *C. difficile* carriage rate of 50%, the highest rate described in CF to date, and demonstrate toxigenic strains and hyper-virulent ribotypes in adult patients with CF, none of whom had symptoms of CDI.

While a recent study reported no significant association between *C. difficile* carriage status and the use of antibiotics in 55 adult patients with CF [11], earlier studies report increasing

Table 4
Association of *Clostridium difficile* with clinical variables.

Variable	CD +ve n = 30 (%, unless otherwise stated)	CD −ve n = 30 (%, unless otherwise stated)	p-Value
Age (median, [IQR])	28 (25.5–38)	26 (23–31)	0.07
Male gender (n = 37)	18 (60)	19 (63)	0.752
Best FEV_1% predicted[a] (%, median, [IQR])	63 (40–79)	64 (49–76)	0.173
PPI use[a] (n = 31)	15 (50)	16 (53)	0.796
Maintenance macrolide therapy[a] (n = 46)	25 (83)	21 (70)	0.953
Pancreatic insufficiency (n = 49)	26 (87)	23 (77)	0.122
Class 1–3 mutation	25 (83)	20 (67)	0.07
Intravenous antibiotic use[b] (n = 11)	4 (13)	7 (23)	0.538
Number of days of intravenous antibiotics[a] (median, [IQR])	14 (0–56)	14 (0–28)	0.440
Number of inpatient days in the 3 years prior to study entry (median, [IQR])	5.5 (0–19.25)	0 (0–22.5)	0.541
BMI (median, [IQR])	24 (20–25.4)	21.3 (18.7–23.2)	0.07
Inhaled prophylactic antibiotics[a]			
Colistin	17 (57)	12 (40)	0.234
Tobramycin	18 (60)	14 (47%)	0.359
All inhaled antibiotics	21 (70)	15 (50%)	0.140

IQR = interquartile range, FEV_1 = forced expiratory volume in 1 s, PPI = proton pump inhibitor, BMI = Body Mass Index, P Ex = pulmonary exacerbation.
[a] During previous 12 months.
[b] At time of sample donation.

C. difficile carriage rates [6–10] possibly reflecting increased cumulative antibiotic exposure as patients with CF survive longer. In our study, the carriage rate of *C. difficile* was 46% (n = 6/13) in patients with CF receiving oral or intravenous antibiotics acutely at the time of sampling. While in earlier studies the carriage rate of *C. difficile* increased in patients receiving long-term or continuous anti-pseudomonal antibiotics [7,8], we did not see the same effect in the small number of patients in our study who were taking short courses (less than 14 days) of anti-pseudomonal antibiotics for treatment of an acute pulmonary exacerbation. This increased *C. difficile* carriage rate in all patients with CF without an increase in carriage rates seen in patients receiving either short-term or continuous antibiotic therapy in our study suggests that novel factors other than antibiotic usage, which are as yet undetermined, may contribute. During this study period, participants had a higher number of home intravenous antibiotics (IVAB) days (median 28, IQR 0–98) than inpatient IVAB days (median 0, IQR 0–22) (data not shown). However, this did not predict the detection of *C. difficile* in the stool of participants. This suggests that nosocomial acquisition of *C. difficile* may not be the major route of acquisition of *C. difficile* in a CF population. While hospital admission is a known risk factor for *C. difficile* carriage [36] and CDI [37] in non-CF populations, this has not been demonstrated in a CF population. Hospital admission may not be as relevant as a risk factor in the CF population as they have regular hospital attendances. In this study, 7 of the 60 samples were submitted by patients who had been admitted to hospital with the remainder submitted by patients who were attending as day-cases in the ambulatory care facility and by patients attending outpatient clinics.

We report the detection of toxigenic *C. difficile* in 32% (n = 19) of our total patient cohort, which corresponds to the detection of toxigenic *C. difficile* in 63% (n = 19/30) of all *C. difficile* isolates detected. The prevalence of toxigenic *C. difficile* in CF varies greatly between previous studies and may reflect different definitions that were used in these studies [6–8,10]. The thirty strains isolated in this study were distributed across 16 distinct ribotypes (RT). Four of the ribotypes detected in this study – RT002, RT014, RT015, and RT078 – appear in the top five most prevalent disease-causing ribotypes reported by the national health protection surveillance centre, with RT014 and the hypervirulent RT078 being consistently the most prevalent ribotypes across the last 4 years of surveillance data [38–41]. This cohort of asymptomatic patients who harbour toxigenic and virulent *C. difficile* in their gut may represent a significant infection risk to any contacts. Further, larger studies are required to examine the interaction between toxin-production, ribotype and pathogenicity in the CF gut.

This study highlights the importance of enforcing strict hand hygiene policy and source isolation of patients with CF. Nosocomial acquisition of a *C. difficile* strain has been demonstrated to be preceded by a documented introduction of that strain to the ward by another asymptomatic ward admission in 16 (84%) of 19 instances in a non-CF population [42]. A further study demonstrated that a prior room occupant with CDI is a significant risk factor for CDI acquisition (HR 2.35), independent of established CDI risk factors such as age, comorbidities, PPI use, and antibiotic exposure [43]. A recent meta-analysis reports that the risk of acquisition of *C. difficile* in a non-CF population from a prior room occupant (OR 2.5) is higher than the risk of acquisition of Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant enterococci (VRE), or Gram negative rods producing Extended-spectrum beta lactamases (ESBL) [44]. The rooms of patients who are colonised with *C. difficile* and do not have diarrhoea (approximately 50% of the CF population in our study) have been shown to be contaminated in 25% of cases [45]. Data from a large surveillance study using molecular subtyping analysis to determine the transmission source in nosocomial CDI demonstrated equal numbers of CDI cases associated with source cases who had symptomatic CDI and those who were asymptomatic carriers [46]. These data suggest that asymptomatic *C. difficile*-colonised new admissions are a potential major source of nosocomial *C. difficile* infections and that asymptomatic CF carriers may represent an important reservoir of *C. difficile* in the hospital environment to any patient. While the practice of isolating patients with CF is born of a desire to provide protective isolation for the patient with CF, it may provide further benefit to the other patients on the same ward, namely the provision of source isolation of an asymptomatic carrier of *C. difficile*. Traditional approaches to control *C. difficile* in the general hospital population, such as the provision of high-profile infection control campaigns, including the enforcement of strict hand hygiene policies appear to be effective in reducing the incidence of CDI [47]. Further campaigns and re-enforcement of hand hygiene policies targeting health care professionals who care for people with CF may reduce the risk of nosocomial transmission of *C. difficile* in this cohort. Equally, the type of hand hygiene is critical with regard to spore-forming organisms such as *C. difficile*. Recent data highlight the lack of sporicidal efficacy of alcohol-based hand washes compared to hand hygiene with soap and water [48]. This study adds weight to the rationale for segregated inpatient and outpatient areas within the hospital for patients with CF. Further studies using a highly discriminatory typing scheme, such as multilocus variable number tandem repeat analysis, to identify and track the transmission of *C. difficile* in patients with CF are required to inform best practice with regard to infection control policy in this area.

This is the first study to examine antibiotic susceptibility of *C. difficile* strains detected in a CF population. A number of studies in a non-CF population have described *C. difficile* strains with reduced susceptibility to metronidazole and vancomycin [49,50]. In our study, all isolates from patients with CF were susceptible to both metronidazole and vancomycin according to the breakpoints defined by the European Committee on Antimicrobial Susceptibility Testing [51], suggesting these antibiotics are highly effective for the treatment of CDI in people with CF pre-lung transplantation.

Antibiotic administration has been shown to be a risk factor for the development of CDI in non-CF cohorts [52], Subjects in this study received an average of 1.6 (SD ± 2.2) intravenous antibiotic (IVAB) 14 day courses spread over an average of 63.8 (SD ± 82.2) days in the 12 months prior to donation of their stool sample. The majority of study participants (77%) were receiving maintenance prophylactic oral macrolide

antibiotics and inhaled antibiotics (60%) in the previous 12 months. Despite this, our study revealed no significant association between the presence of *C. difficile* and the use of short-term intravenous anti-pseudomonal antibiotics and/or oral continuous macrolide therapy over the previous 12 months. Given the limited systemic absorption of inhaled antibiotics, it is not surprising that we found no association between the presence of *C. difficile* and any inhaled antibiotics.

The effect of CFTR modulator therapy on the gut microbiota and, specifically, the carriage rate of *C. difficile* is unknown. During the study period, 13 patients commenced Ivacaftor therapy. 4 of these had commenced Ivacaftor prior to donating a stool sample; 1 patient commenced Ivacaftor 1 day prior to stool sampling, 1 patient commenced Ivacaftor 7 days prior to stool sampling with 2 further patients taking Ivacaftor for 6 months and 13 months respectively at the time of stool sampling. None of the patients taking Ivacaftor at the time of stool sampling were *C. difficile* positive.

The findings of this study provide a number of challenging clinical dilemmas for the CF clinician. No consensus exists as to whether patients with CF should be screened for the presence of *C. difficile*, whether attempted eradication of *C. difficile* is beneficial and if so, what regimen should be used and when it should be initiated. Screening for *C. difficile* in the absence of symptoms is not common practice, yet our data suggest that this strategy will result in detection of many cases of *C. difficile* carriage as the majority of patients with CF will remain asymptomatic despite the presence of toxigenic, virulent *C. difficile* in their gut. However, an immediate benefit of attempted eradication of *C. difficile* in asymptomatic patients with CF is unclear given that our data shows no association between the presence of *C. difficile* and clinical markers of disease severity. No data exist regarding the optimal eradication regime for *C. difficile* in asymptomatic patients with CF. While our study demonstrates the in-vitro efficacy of metronidazole and vancomycin against all isolates, data examining an asymptomatic non-CF *C. difficile* carrier population suggest that eradication therapy with metronidazole or vancomycin may not be effective [53]. While traditional approaches for management of CDI, such as the use of probiotics [54] or faecal microbiota transplantation [55], have shown benefit in a non-CF population, these are untested in a CF population and may provide a novel mechanism of addressing this issue. Three studies suggest a benefit to the use of probiotics in a paediatric CF population, demonstrating a reduction in pulmonary exacerbation rates [18,19,56], and one study demonstrating a reduction in hospital admission rate, improved lung function and improved body weight with daily probiotic use [18]. Eradication therapy may be particularly beneficial in the immediate pre-transplant period in an attempt to reduce the incidence of reported severe and sometimes fatal *C. difficile* colitis experienced in the post-transplant period in this cohort [28–30]. While *C. difficile* carriage is not an exclusion criterion for lung transplant, eradication of *C. difficile* in patients awaiting transplant may prove beneficial as the presence of *C. difficile* may act as a relative deterrent to transplant programs in patient selection. Finally, eradication of *C. difficile* from the CF gut may allow a more diverse and, therefore, more healthy GI flora. Given the emerging evidence for the relationship between gut health and lung health in patients with CF [20,21] – the "Lung–Gut Axis" – eradication of *C. difficile* from the gut may result in improved lung health in this cohort.

6. Limitations

This study is a single-centre study with retrospective clinical data collection. We examine 60 patients with CF from a single geographical area, which may not represent the patterns of *C. difficile* carriage seen in other geographical areas. There is no validated CF-specific GI symptom assessment tool and we relied on patient-reporting of symptoms suggestive of CDI. We did not perform sampling of the hospital environment or of stool from other patients on the same ward who developed diarrhoeal illnesses subsequent to visits to the hospital by participants in this study. The use of multilocus variable number of tandem repeats analysis genotyping on these samples may demonstrate nosocomial acquisition of C difficile from an asymptomatic carrier with CF. Another limitation is that we did not collect data regarding shared attendance at hospital that may have demonstrated the potential for cross infection.

7. Conclusions

C. difficile carriage in patients with CF is common and appears to be increasing, with half of the adults in this study carrying *C. difficile* in their gut including toxigenic and hyper-virulent strains without symptoms of CDI. Studies examining *C. difficile* transmission patterns among patients with CF and other hospitalised patients along with studies examining the effects of eradication of *C. difficile* on health outcomes in both pre- and post-transplant patients with CF are required to provide further insight into this area.

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Conflict of interest statement

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Contributorship statement

BJP, MCR, CS, RPR, DGB, FS, CH and MH were involved in the planning of the study. BJP, MCR, CS, RPR, DGB, MH, DM, CF, MM, JAE, IS and C Shortt were involved in the conduct of the study. DGB, MH and BJP produced the initial draft of the manuscript with all authors contributing to the final submitted version.

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