Clostridium difficile infection : epidemiology, complications and recurrences

Bauer, M.P.

Chapter 3

Patients with cystic fibrosis have a high carriage rate of non-toxigenic *Clostridium difficile*

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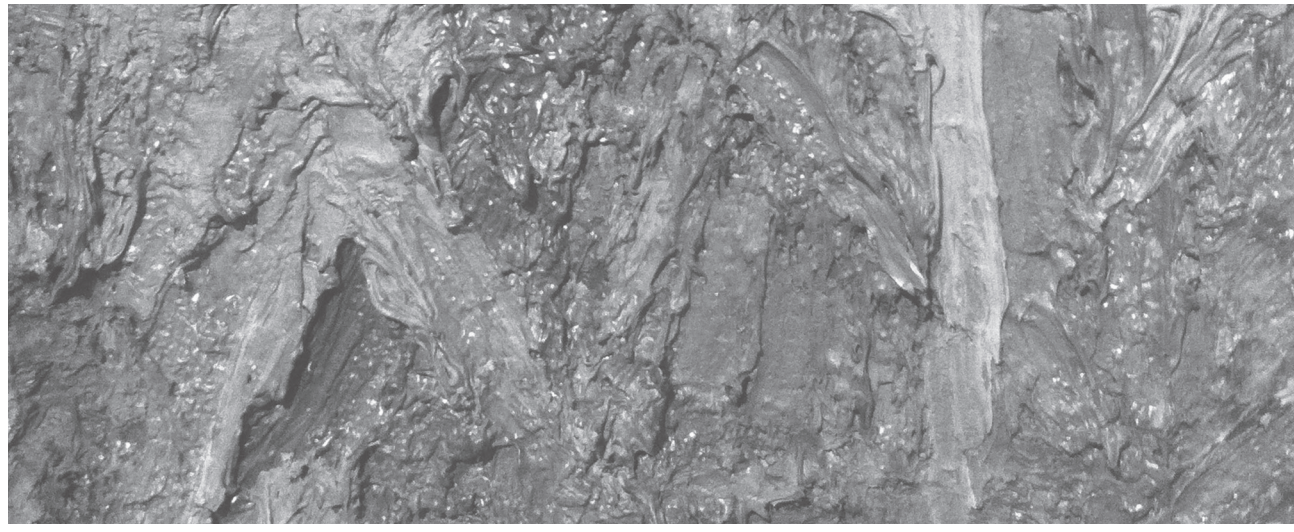

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Abstract

Thirty-year-old observations report frequent asymptomatic *Clostridium difficile* carriage among cystic fibrosis (CF) patients. In this case-control study, we found more carriers among CF patients than controls (47% versus 11%), but most strains carried by CF patients were non-toxigenic (77% versus 17%). Among CF patients, carriers were younger with more-severe pulmonary disease than non-carriers. Strains belonged to multiple PCR-ribotypes, suggesting that these CF patients did not acquire strains from each other.

Clostridium difficile infection (CDI) is an important cause of diarrhea and colitis. The most important risk factors are exposure to healthcare institutions and the use of antibiotics. Other associated factors are advanced age, severe comorbidity, decreased humoral immunity against *C. difficile* toxins and the use of proton pump inhibitors. Intestinal colonization with *C. difficile* may lead to disease, but also to asymptomatic carriage. The role of asymptomatic carriers in the spread of CDI is unclear as yet [1], as is the reason why some become carriers and others develop disease.

Several studies have suggested that patients with cystic fibrosis (CF) are often asymptomatic carriers of *C. difficile* [2-4], not surprising given their frequent use of antibiotics and exposure to hospitals. However, these observations were made in the 1980s, when the incidence of CDI was lower than now and epidemic strains such as PCR ribotype 027 had not yet emerged. Furthermore, the strains CF patients carry have not been characterized with molecular methods and predictors of *C. difficile* carriage among CF patients have not been investigated. Lastly, why CF patients apparently seldom develop disease remains unclear. Filling in these knowledge gaps may provide insight in CDI epidemiology and pathogenesis and have implications for infection prevention. In this case-control study, we sought to confirm earlier observations of frequent *C. difficile* carriage in CF patients, to characterize their *C. difficile* strains using molecular methods, to compare the aforementioned risk factors for CDI in this group with a control group and to identify predictors of *C. difficile* carriage in CF patients.

From June 2012 through November 2012, all adult CF patients monitored at Erasmus Medical Center, Rotterdam, a national CF center, were asked to participate in the study. The only exclusion criterion was failure to give informed consent. Inpatients submitted a stool sample on the ward, which was transported to Leiden University Medical Center the same day. Outpatients sent a stool sample by mail, which usually arrived the next day. Stool samples were cultured for *C. difficile* using selective media and the alcohol shock method [5]. Strains were characterized by PCR-ribotyping [6] and PCRs for toxin genes (TcdA, TcdB and binary toxin genes CdtA and CdtB) [5, 7, 8]. Patients described their bowel movements in a diary [9]. Clinical and epidemiological information was collected from patient charts.

As controls, we asked all patients present on 10 separate days between March 1st 2011 and September 30th 2011 on the internal medicine ward of Bronovo Hospital, The Hague, a general hospital with 815 beds, to participate in the study.

The distributions of continuous variables were compared using a Mann-Whitney *U* test. Pulmonary function test results were log-normalized for comparison and compared by *t* test. For associations between categorical variables, odds ratios (ORs) with 95% confidence intervals (95% CI) were calculated. IBM SPSS Statistics 20.0 software was used for the calculations.

Fifty-five CF patients and 108 controls submitted a stool sample. Twenty-six (47%) of CF patients were carriers versus 12 (11%) of controls (OR: 7.17; 95% CI: 3.22 – 16). Only 6 (23%) strains from CF patients were toxigenic, contrary to 10 (83%) strains from controls. Strains in both groups belonged to various PCR ribotypes (CF patients: 009, 010 (7 patients), 012 (2), 035, 039 (5), 046, 078, 097, 140, 151, 169, 207, unknown (3); controls: 018, 026, 043, 054, 076 (2), 081, 140, 142, unknown (2), 1 strain was not available). In only two strains, belonging to PCR ribotype 078 and an unknown ribotype, both from CF patients, genes for binary toxin were present.

None of 36 carriers had diarrhea (a mean of three watery bowel movements during three consecutive days), as opposed to five of 115 non-carriers; information was incomplete in 12 patients.

Among controls, the only statistically significant association was heart failure as defined in the Charlson comorbidity index [10] (Table 1).

CF patients had received more antibiotics than controls. Among CF patients, surprisingly, carriers were younger than non-carriers (Table 2). Also, carriers had worse pulmonary function parameters. Carriage was associated with severe (class I) mutations in the CFTR gene. Continuous variables were dichotomized using the median of the whole population as a cut-off. Because of an inverse relationship between age and pulmonary function, the odds ratios for pulmonary function parameters were adjusted for age by logistic regression analysis to correct for confounding. Age under 31 and FEV1 under 65% of predicted were significantly associated with carriage.

Strengths of this study were the detailed data, including daily defined antibiotic doses, pulmonary function tests and CFTR mutations, and the molecular characterization of *C. difficile* strains. A weakness of this study is the small number of patients. Furthermore, a comparison of CF patients with any other patient group is necessarily flawed because of differences in age and comorbidities. We tried to compensate for this by detailed documentation of risk factors for *C. difficile* carriage.

In the three studies from the 1980s, carriage rate among CF patients receiving antibiotics ranged from 22% to 50% versus 0% among those not receiving antibiotics [2-4]. Most of the colonized patients did not have diarrhea. Our finding of a high asymptomatic carriage rate is compatible with these studies, but only one study found a similarly low proportion of toxigenic strains. The association between carriage and more-severe pulmonary disease can probably be explained by higher antibiotic consumption. The association with younger age might tentatively be explained by a higher exposure to (non-toxigenic) strains circulating in the community.

Colonization with non-toxigenic *C. difficile* may protect against colonization with toxigenic strains [11] and may partially explain why CF patients seldom develop disease. Non-toxigenic strains might be less efficient at establishing long-term carriage than toxigenic *C. difficile* [12, 13]. We hypothesize that, due to differences in

colonic mucus or microbiome [14], non-toxigenic strains can colonize CF patients more efficiently than non-CF patients. The questions remain how non-toxigenic *C. difficile* strains can establish durable colonization in CF patients, and whether other factors than colonization by non-toxigenic strains protect CF patients from CDI.

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Transparency declaration

The authors declare no conflicts of interest.

Table 1 Characteristics of *C. difficile* carriers and non-carriers among controls.

	Carriage			No carriage			
Continuous variables	N	Median	IQR	N	Median	IQR	*P*
Age [years]	12	76	69 – 83	96	70	56 – 82	0.257
Total defined daily doses of antibiotics consumed in the previous 3 months	7	0	0 – 7	81	0	0 – 4	0.648
Charlson comorbidity index	12	3	0 – 4	96	2	0 – 3	0.531
Duration of current admission [days]	12	5	3 – 7	95	4	2 – 6	0.186

Categorical variables	N	%	N	%	OR	95%CI
Male sex	12	42	96	48	0.78	0.23 – 2.62
Use of antibiotics in the previous 3 months	12	67	96	44	2.57	0.73 – 9.12
Use of ceftazidime in the previous 3 months	12	8	96	1	8.64	0.51 - 148
Use of ciprofloxacine in the previous 3 months	12	33	93	15	2.82	0.75 – 11
Use of any immunosuppressant in the previous 3 months	12	17	95	19	0.86	0.17 – 4.25
Use of proton pump inhibitor in the previous 3 months	12	50	96	58	0.71	0.22 – 2.37
Hospital admission in the previous 3 months	12	33	96	20	2.03	0.55 – 7.44
Comorbidity as defined by Charlson index:						
History of myocardial infarction	12	25	96	15	1.95	0.47 – 8.11
Congestive heart failure	12	42	96	14	**4.56**	**1.26 – 17**
Peripheral vascular disease	12	8	96	5	1.66	0.18 – 15
Cerebrovascular disease	12	8	96	9	0.88	0.10 – 7.61
Dementia	12	0	96	2		
Chronic pulmonary disease	12	25	96	15	1.95	0.47 – 8.11
Rheumatologic disease	12	0	96	3		
Peptic ulcer disease	12	0	96	4		
Diabetes mellitus without microvascular disease	12	17	95	22	0.71	0.14 – 3.47
Diabetes mellitus with microvascular disease	12	8	96	4	2.09	0.21 – 20
Hemiplegia or paraplegia	12	0	95	1		
Renal disease	12	8	96	10	0.78	0.09 – 6.71
Non-hematologic malignancy	12	0	96	24		
Leukemia	12	8	96	0		
Lymphoma	12	8	96	2	4.27	0.36 – 51
Liver cirrhosis with portal hypertension or hepatic coma	12	17	96	0		
Metastasized non-hematologic malignancy	12	0	96	13		
HIV infection	12	0	96	0		

N = number of patients for whom information was available. IQR = interquartile range. OR = odds ratio. Results printed in boldface have reached statistical significance ($P < 0.05$).

Table 2 Characteristics of *C. difficile* carriers and non-carriers among cystic fibrosis patients

	Carriage			No carriage			
Continuous variables	N	Median	IQR	N	Median	IQR	*P*
Age	26	26	21 – 39	29	35	26 – 45	0.066
Total defined daily doses of antibiotics consumed in the previous 3 months	22	75	45 – 90	25	64	0 – 71	0.116
Forced expiratory flow between 25 to 75% of vital capacity [% of predicted]	23	16	9 – 52	28	29	12 – 58	0.227
Residual volume/ total lung capacity ratio [% of predicted]	21	115	84 – 146	28	91	80 – 114	0.054
Forced expiratory volume in 1 second [% of predicted]	23	54	32 – 80	29	73	52 – 89	0.085
Forced expiratory volume in 1 second/ vital capacity ratio [% of predicted]	23	65	60 – 80	29	74	59 – 93	0.378

Categorical variables	N	%	N	%	OR	95%CI
Age under 31	26	62	29	53	2.62	0.88 – 7.78
Age under 31, adjusted for forced expiratory volume in 1 second <65% of predicted					**3.98**	**1.08 – 14.67**
Forced expiratory flow between 25 to 75% of vital capacity <23% of predicted	23	57	28	43	1.73	0.57 – 5.28
Residual volume/ total lung capacity ratio ≥ 97% of predicted	21	67	28	46	2.31	0.71 – 7.45
Forced expiratory volume in 1 second <65% of predicted	23	65	29	38	3.07	0.98 – 9.59
Forced expiratory volume in 1 second <65% of predicted, adjusted for age less than 31					**4.61**	**1.25 – 17.03**
Forced expiratory volume in 1 second/ vital capacity ratio < 69% of predicted	26	54	29	38	1.91	0.65 – 5.60
Use of antibiotics in the previous 3 months	26	85	29	69	2.48	0.66 – 9.31
Use of ceftazidime in the previous 3 months	26	19	28	4	6.43	0.70 - 59
Use of azithromycin in the previous 3 months	26	73	29	48	2.91	0.94 – 9.02
Use of any immunosuppressant in the previous 3 months	23	26	29	14	2.21	0.54 – 9.01
Use of proton pump inhibitor in the previous 3 months	25	48	29	41	1.31	0.45 – 3.84
Admitted to hospital at the time of collection of stool sample	26	15	29	7	2.46	0.41 – 14.67
Hospital admission in the previous 3 months	26	12	29	7	1.76	0.27 – 11.5
Chronic sinusitis	25	52	28	32	2.29	0.75 – 6.98
Exocrine pancreatic insufficiency	26	85	29	72	2.10	0.55 – 8.01
Endocrine pancreatic insufficiency	26	27	29	28	0.97	0.29 – 3.18
History of meconium ileus or distal obstructive syndrome	26	12	29	10	1.13	0.21 – 6.16
Liver cirrhosis	26	8	29	10	0.72	0.11 – 4.70
Homozygous for ΔF508	22	50	29	48	1.07	0.35 – 3.25
Compound heterozygous for ΔF508	22	41	29	38	1.13	0.37 – 3.52
1 class I and 1 class II mutation	22	18	24	4	5.11	0.53 – 50
1 class I and 1 class III mutation	22	5	24	0		
1 class I and 1 class V mutation	22	5	24	0		
1 class I and second unknown mutation	22	0	25	8		
Any class 1 mutation	22	27	25	8	4.31	0.77 - 24
2 class II mutations	22	50	24	63	0.60	0.19 – 1.94
1 class II and 1 class IV mutation	22	0	24	4		
1 class II and 1 class V mutation	22	23	24	25	0.88	0.23 – 3.44
2 class IV mutations	22	0	24	4		

N = number of patients for whom information was available. IQR = interquartile range. OR = odds ratio. Results printed in boldface have reached statistical significance ($P < 0.05$).

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