

Clostridium difficile infection : epidemiology, complications and recurrences

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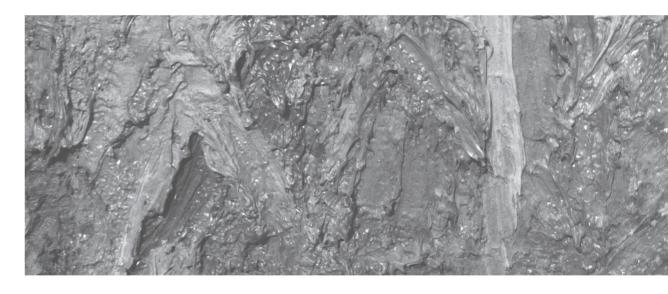
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Chapter 3

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

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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	le l		No carriage	age		
Continuous variables	z	Median	IQR	z	Median	IQR		Д
Age [years]	12	92	69 – 83	96	70	56 – 82		0.257
Total defined daily doses of antibiotics consumed in the previous 3 months	_	0	2 - 0	81	0	0 – 4		0.648
Charlson comorbidity index	12	က	0 – 4	96	Ø	0 – 3		0.531
Duration of current admission [days]	12	Ŋ	3 – 7	92	4	2 - 6		0.186
Categorical variables	Z		%	Z		%	OR	95%CI
Male sex	12		42	96		48	0.78	0.23 – 2.62
Use of antibiotics in the previous 3 months	12		29	96		44	2.57	0.73 - 9.12
Use of ceftazidime in the previous 3 months	12		8	96		-	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	92		19	0.86	0.17 - 4.25
Use of proton pump inhibitor in the previous 3 months	12		50	96		28	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		80	96		6	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		က		
Peptic ulcer disease	12		0	96		4		
Diabetes mellitus without microvascular disease	12		17	92		22	0.71	0.14 - 3.47
	!			}		ł	-	
Diabetes mellitus with microvascular disease	12		ω	96		4	5.09	0.21 - 20
Hemiplegia or paraplegia	12		0	92		_		
Renal disease	12		8	96		10	0.78	0.09 - 6.71
Non-hematologic malignancy	12		0	96		24		
Leukemia	12		80	96		0		
Lymphoma	12		8	96		2	4.27	0.36 - 51
Liver cirrhosis with portal hypertension or hepatic	12		17	96		0		
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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	ge Ge		No carriage	de		
Continuous variables	z	Median	IOR	z	Median	IQR		Ь
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	53	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	53	73	52 – 89		0.085
Forced expiratory volume in 1 second/ vital capacity ratio [% of predicted]	23	92	08 - 09	59	74	59 - 93		0.378
Categorical variables	Z		%	Z		%	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		27	28		43	1.73	0.57 – 5.28
Residual volume/ total lung capacity ratio ≥ 97% of predicted	21		29	28		46	2.31	0.71 – 7.45
Forced expiratory volume in 1 second <65% of predicted Forced expiratory volume in 1 second <65% of predicted, adjusted for age less than 31	23		92	59		38	3.07 4.61	0.98 – 9.59 1.25 – 17.03
Forced expiratory volume in 1 second/ vital capacity ratio < 69% of predicted	26		54	53		38	1.91	0.65 - 5.60
Use of antibiotics in the previous 3 months	56		85	53		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	59		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	53		41	1.31	0.45 - 3.84
Admitted to hospital at the time of collection of stool sample	56		15	53		7	2.46	0.41 – 14.67
Hospital admission in the previous 3 months	56		12	53		7	1.76	0.27 - 11.5
Chronic sinusitis	52		52	8 8		32	2.29	0.75 - 6.98
Exocrine pancreatic insufficiency Endocrine pancreatic insufficiency	9 7 8		85	R 8		7.7.	2.10	0.55 - 8.01
History of meconium ileus or distal obstructive syndrome	2 6		12	3 6		2 2	1 0.7	0.21 - 6.16
Liver cirrhosis	26		! ∞	3		2 2	0.72	0.11 – 4.70
Homozygous for Δ F508	22		50	53		48	1.07	0.35 - 3.25
Compound heterozygous for $\Delta F508$	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		2	24		0		
1 class I and 1 class V mutation	22		22	24		0		
1 class I and second unknown mutation	5 5		0 0	K 5		ω (0	0
Any class I mutation 2 class II mitations	22 6		27	57 S		χ Σ	18.4	0.77 - 24
1 class II and 1 class IV mutation	22		8 0	24		3 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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