Prevalence, Duration, and Risk Factor Analysis for Asymptomatic Carriage of *Clostridium difficile* Among Healthy Subjects in Pittsburgh, Pennsylvania

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ABSTRACT

Previous studies suggest that 7-15% of healthy adults are carriers of toxigenic *Clostridium difficile* (CD). We recruited healthy adults with no recent history of diarrhea; persons who reported recent hospitalization, history of CD infection (CDI), or employment in a health care facility were excluded. Participants provided epidemiologic data, recorded an online food diary, and submitted a stool specimen. Subjects positive for CD by toxigenic anaerobic culture were asked to submit additional specimens. One hundred six (81%) of 130 subjects submitted specimens, 7 of whom (6.6%) were found to be CD carriers. Six subjects submitted additional specimens. Stool from 2 individuals yielded CD on 2 successive occasions 1 month apart. Epidemic lineages of CD (ribotypes 027, 078) were not observed. Demographics, diet, health care facility exposure, antibiotic exposure, and exposure to persons with CDI were not significantly associated with CD carriage. The prevalence of CD carriage in this healthy cohort is concordant with prior estimates. CD carriers may be important reservoirs for CDI and may test falsely positive for CDI when evaluated for community acquired diarrhea.

INTRODUCTION

Toxin-producing CD is present in 5-15% of healthy, non-U.S. populations.[1-3] The potential sources for CD in healthy carriers are unknown.

The degree to which CD-carrying healthy persons serve as transmission reservoirs is unknown, but their potential role in incident CDI is plausible based on current evidence:

- Up to 30% of CDI is community acquired.[4]
- Mathematical models suggest that the rate and molecular diversity of HA-CDI that could not be supported by nosocomial transmission of infecting CDI strains alone.[5]

Our study sought to achieve the following objectives:

- Describe the epidemiology of non-hospitalized adults with asymptomatic CD carriage
- Examine the frequency and distribution of CD subtypes isolated from asymptomatic individuals
- Determine the quantitative abundance of CD in the stool of asymptomatic carriers and the frequency with which carriers test positive for CD using commercially-available loop-mediated isotheral amplification tests

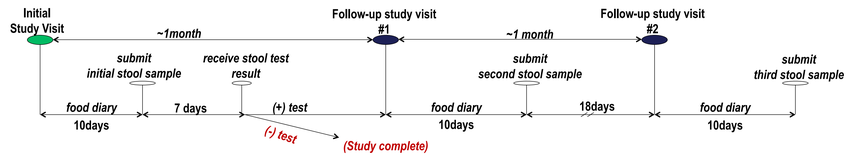
METHODS

Inclusion criteria:
- Age ≥ 18 years
- Able to keep online food diary
- Independent resident of Allegheny county, PA (USA)

Exclusion criteria:
- Employment in a healthcare setting
- Presence of an ostomy or partial/total colectomy
- History of CDI
- History of hospitalization in the previous 12 months
- History of diarrheal illness in the preceding 3 months
- Chronic constipation (<2 stools/week)
- Chronic diarrhea due to irritable bowel syndrome, inflammatory bowel disease, or other chronic medical conditions

METHODS (cont.)

Timeline of ISCAP subject participation



Microbiologic methods

- All stool was shipped to the lab at ambient temperature and stored at -80 C.
- Each stool specimen was cultured for CD using 4-quadrant direct plating and broth amplification.[6]
- *tcdC* genotyping and *lok1/lok3* PCR was used to infer toxigenicity of isolates.[7]
- *tcdC* genotypes were assigned using PubMLST database (http://pubmlst.org/cdifficile)
- Positive specimens underwent loop-mediated isothermal amplification (*illumi*gene *C. difficile*, Meridian Bioscience, Cincinnati, OH) and were inoculated onto solid media for quantitative culture.

Analysis

- Descriptive statistics of the sample including frequency tables were examined
- Epidemiologic data were analyzed to identify characteristics associated with CD colonization (SAS™ Version 9.2, SAS Institute, NC, USA)

RESULTS

- One hundred six (81%) of 130 enrolled participants submitted stool specimens.
- Seven (6.6%) of 106 participants were CD carriers. There was no recovery of non-toxigenic CD.

Table 1. Association of baseline participant characteristics with CD carriage risk. Most participants were omnivorous females aged <35years. Demographics, diet, health care facility exposure, antibiotic exposure, and exposure to persons with CDI were not significantly associated with CD carriage. Participants who reported pet exposure and frequent public restroom use were less likely to carry CD, but this association did not reach statistical significance

Participant characteristics	N (% of total)	*C.difficile* colonization risk: #colonized/ total (%)	OR	CI	p-value
Age 18-24	37 (35%)	4/37 (11%)	baseline	–	0.42
Age 25-34	28 (26%)	1/28 (3.6%)	0.31	(0.01, 3.38)	
Age 35-54	21 (20%)	2/21 (9.5%)	0.87	(0.07, 6.75)	
Age 55+	20 (19%)	0/20 (0.0%)	0.33**	(0.00, 2.02)	
Male	37 (35%)	2/37 (5.4%)	0.73	(0.07, 4.77)	1
Female	69 (65%)	5/69 (7.2%)			
Omnivore	100 (94%)	6/99 (6.1%)	0.32	(0.03,17.58)	0.69
Vegetarian	6 (6%)	1/6 (17%)			
Consumes raw beef	13 (12%)	2/13 (15%)	3.15	(0.27, 22.36)	0.41
Avoids raw beef	93 (88%)	5/93 (5.4%)			
Consumes raw seafood	59 (56%)	5/59 (8.5%)	2.07	(0.32, 22.72)	0.65
Avoids raw seafood	47 (44%)	2/47 (4.3%)			
Pet exposure	42 (40%)	0/42 (0.0%)	0.14**	(0.00, 0.74)	0.05
No pet exposure	62 (58%)	7/62 (12%)			
Physician office exposure ≤ 90d	53 (50%)	5/53 (9.4%)	1.09	(0.63, 2.01)	0.88
Physician office exposure > 90d	53 (50%)	2/53 (3.8%)			
Antibiotic exposure ≤ 90d	12 (11%)	2/12 (17%)	3.50	(0.30, 25.17)	0.36
No antibiotic exposure ≤ 90d	94 (89%)	5/94 (5.3%)			
Regular contact with children 0-4yrs	17 (16%)	0/17 (0.0%)	0.51**	(0.00, 2.78)	0.56
No regular contact with children 0-4yrs	88 (83%)	7/88 (8.0%)			
Public restroom use < 1 time/week	19 (18%)	3/19 (16%)	baseline	–	0.21
Public restroom use 1-6 times/week	48 (45%)	3/48 (6.3%)	0.36	(0.06,2.98)	
Public restroom use ≥ 7 times/week	38 (36%)	1/38 (2.6%)	0.15**	(0.00,2.02)	
Known exposure to CDI	1 (1%)	0/1 (0.0%)	3.76	(0.00,16.39)	1
No known exposure to CDI	105 (99%)	7/105 (6.7%)			

**Median unbiased estimate

RESULTS (cont.)

Table 2. Stool testing characteristics of CD carriers. Six (86%) CD carriers submitted >1 stool specimen. Stool from 2 (29%) individuals yielded CD on 2 successive occasions 1 month apart. The *illumi*gene assay was positive for 3 (43%) stool specimens. Stool from each unique participant yielded a different *tcdC* genotype. Epidemic lineages of CD were not observed.

Participant	Visit	CD culture	quantitative culture (cfu/g stool)	*illumi*gene	*tcdC*
1	1	POS	2.7×10^3	neg	*tcdC 5*
	2	NEG	-	-	-
	3	NEG	-	-	-
2	1	POS	NG	neg	*tcdC 20*
	2	NEG	-	-	-
3	1	POS	8.7×10^3	neg	*tcdC 19*
	2	POS	4.9×10^4	pos	*tcdC 19*
4	1	POS	3.0×10^4	pos	*tcdC 14*
	2	NEG	-	-	-
	3	NEG	-	-	-
5	1	POS	NG	neg	*tcdC 53*
6	1	POS	8.0×10^4	neg	*tcdC 3*
	2	NEG	-	-	-
7	1	POS	1.1×10^3	neg	*tcdC 10*
	2	POS	tntc	pos	*tcdC 10*

CONCLUSIONS

- The prevalence of CD colonization in this healthy cohort - 6.6% - is concordant with prior estimates in non-US populations.
- Healthy CD carriers may be important reservoirs for CDI.
- CD carriers may falsely test positive for CDI when evaluated for community acquired diarrhea.
- Demographics, diet, health care facility exposure, antibiotic exposure, and exposure to persons with CDI were not significantly associated with CD carriage.
- A larger sample size may be necessary to identify risk factors for CD carriage among healthy individuals.

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