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A Case-Control Study To Assess Risk Factors For Community-Associated Clostridium Difficile Infection

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ABSTRACT

INTRODUCTION: *Clostridium difficile* infection (CDI), initially thought to be acquired in the hospital, has occurred often in persons not recently hospitalized. Community-associated CDI (CA-CDI) may affect healthy individuals who lack the traditional risk factors for health care-associated CDI (HA-CDI). Risk factors for CA-CDI have yet to be fully identified.

OBJECTIVES: The objective of this study was to assess the risk factors for CA-CDI in the small Connecticut sample of a larger multi-state case-control study being conducted by the Emerging Infections Programs, and to help inform analysis of the larger study.

METHODS: A matched case-control study was conducted of consenting individuals. Cases had positive cultures for *C. difficile* and underwent chart review to confirm as community-associated. Controls were persons identified through random digit dialing without prior CDI and were age and county-matched to cases. Participants were enrolled from November 2014 through March 2015. Interview questions were asked regarding potential exposures in the preceding 2-12 weeks. Matched and conditional logistic regression analyses were conducted. An unmatched analysis was performed to stratify leading *C. difficile* exposures by antibiotic use.

RESULTS: Of the cases, 16 (72.3%) reported exposure to a medical setting within two weeks of symptom onset. Nine (56.3%) of these reported having taken an antibiotic within four weeks of symptom onset. In the matched analysis, exposures in the twelve weeks prior to symptom onset significantly increasing the odds of CA-CDI included antibiotic use (OR=12.00, 95% CI 1.78-512.97), any medical/dental procedure (OR=10.00, 95% CI 1.42-433.98), any medical care/visit (OR=9.61, 95% CI 1.87-undefined), and use of untreated tap water (OR=3.50, McNemar's). In the unmatched analysis, those who did not take antibiotics and had a household member who wore diapers had a significantly increased odds of CA-CDI (OR=116.67, 95% CI 1.22-5.88).

CONCLUSION: Common risk factors for CA-CDI included exposure to a health care facility, having a medical/dental procedure, and taking an antibiotic in the twelve weeks prior to symptom onset. Antibiotics taken in proximity to time of exposures appear to facilitate them. Significant exposures should be further investigated in the larger study. While the small sample size (N=42) of this study limited the statistical power, the analysis provides a preview of what may be seen in the results of the larger multi-state study to which this sample contributes.

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INTRODUCTION

Clostridium difficile was first recognized as the causative agent of some diarrheal infections in the late 1970s (Sepkowitz, 2013). *C. difficile* infection (CDI) was thought to be precipitated by antibiotic use, particularly use of broad-spectrum antibiotics such as clindamycin, ampicillin, cephalosporins, and fluoroquinolones (Sepkowitz, 2013). Initially, cases were believed to be mostly acquired in the hospital setting and to be associated with exposure to the antibiotics most commonly used in hospitals.

Surveillance for CDI began in some areas in the 1990s and since then, the incidence of CDI has increased steadily. From 1991 to 2003 in Quebec, CDI incidence increased from 35.6 to 146.3 cases per 100,000 persons, while CDI incidence in those 65 years and older increased from 102.0 to 866.5 cases per 100,000 persons (Pepin, 2004). This increase in CDI in Quebec was seen elsewhere as well. From 1996 to 2003, U.S. National Hospital Discharge Survey Data confirmed that the incidence of CDI increased nearly two-fold (McDonald, 2006). Today in the United States approximately 453,000 cases of CDI occur each year (Lessa, 2015), costing $8.2 billion dollars in health care expenses (Lucado, 2006), and are associated with 29,300 deaths (Lessa, 2015). CDI morbidity can be high, in part because disease recurrence occurs in about 83,000 cases annually (Lessa, 2015). Furthermore, recurrences may occur multiple times over months or years. CDI can severely damage the digestive tract, leading to pseudomembranous colitis (PMC), which often occurs in fatal cases (Siemann, 2000).

Surveillance has shown that the increase in incidence of CDI in the United States seen over the past 15 years has been associated with the emergence of more toxigenic strains of *C. difficile*, such as the NAP1/027/B1 strains. These “hypervirulent” strains produce B1, the binary *C. difficile* toxin (CDT) (Viswanathan, 2010). B1 ribosyl-transferase activity is known to inactivate host-cell signaling pathways. This results in disruptions of the cytoskeletal arrangement, and ultimately cell apoptosis (Viswanathan, 2010).

The NAP1 strain has been implicated as the causative agent of many hospital outbreaks of CDI (Johnson, 1999; McDonald, 2005; Loo, 2005). The strain, however, was also isolated from community-associated CDI (CA-CDI) cases beginning in 2005. This discovery was followed by the release of an MMWR about the increasingly dangerous CDI (MMWR, 2005). The strain was capable of causing severe cases of CA-CDI even in otherwise healthy individuals, in contrast to the belief that CDI was mostly hospital-associated (MMWR, 2005). A study that

year in Paris found that the hypertoxigenic CDI strain actually caused a higher proportion of infection in CA-CDI cases than in hospital-acquired CDI cases (Barbut, 2005). Over time, NAP1/B1/027 strains have rapidly replaced other, more common strains of *C. difficile* in the community (Hunt, 2013). It is believed that the increased incidence of CDI in community settings may in large part be due to the ability of the NAP1/B1/027 strains to more easily affect populations traditionally considered healthy.

Risk factors for healthcare-associated CDI (HA-CDI) have been well studied. These include increasing age, presence of underlying conditions, gastrointestinal procedures, use of nasogastric tube, acid-reducing medications, ICU stay, longer length of hospital stay, longer duration of antibiotic use, and use of multiple antibiotics (Bignardi, 1998). A broader population of susceptible persons without these risk factors for CDI, including those of younger age, fewer comorbidities, fewer exposures to healthcare settings and fewer having had antibiotics, but with exposure to infants and/or household contacts with CDI, are now recognized as being at risk for CDI as well (Chitinis, 2013). Research suggests that as a greater proportion of this population is colonized with *C. difficile*, antibiotics used in the community setting may precipitate symptomatic CA-CDI (Chitinis, 2013). Yet, many cases considered CA-CDI report health care system exposure, even without hospitalization, in the three months prior to the onset of symptoms. This raises the issue of whether some CA-CDI cases, as currently defined, are truly HA-CDI cases.

The 2005 MMWR that alerted the nation to the NAP1 strain in CA-CDI cases called for "the need for surveillance to better understand the changing epidemiology of CDAD [*C. difficile*-associated disease]" (MMWR, 2005). In 2009, Emerging Infections Programs (EIPs) in seven states began surveillance programs for CDI to monitor these changing patterns of disease. EIP data has been used to assess the burden of CDI in the United States, monitor changes in the disease over time, and determine which strains are responsible for infection. The Centers for Disease Control and Prevention (CDC) used surveillance data from 2009 to 2011 to assess some of the risk factors for CA-CDI. While the information gathered from this study has been informative and led to a number of publications, the authors cited the lack of a control group with which to compare the cases as a major limitation to the data collected (Chitinis, 2013). Studies assessing risk factors for CA-CDI have been otherwise limited.

To further our understanding of CA-CDI, a case-control study was designed and coordinated by the CDC again using the EIPs. This multi-state study is attempting to explore some of the possible risk factors for CA-CDI suggested by earlier EIP surveillance data. These include exposure to infants, household contacts, exposure to outpatient medical facilities, diet, regular use of anti-depressants and/or proton pump inhibitors (PPIs), and household water source. Additionally, the study seeks to increase the evidence base for CA-CDI in order to update our understanding of CA-CDI and prevent infections in the future.

METHODS

CDI Surveillance

The Connecticut EIP began active laboratory-based and population-based surveillance for CDI in 2009. EIP surveillance identifies positive *C. difficile* toxin enzyme immunoassay (EIA) or positive *C. difficile* molecular assay (e.g. PCR) on stool specimens from persons with illness from all inpatient and outpatient clinical laboratories in New Haven County. CDI is defined as a positive CDT EIA or a positive *C. difficile* molecular assay on an incident stool specimen from a New Haven County resident greater than 1 year old who did not have a positive assay in the 8 weeks prior. After identification of a patient with CDI, medical records were reviewed to classify patients without overnight stay in a hospital or long-term care facility as having CA-CDI. Cases were defined as CA-CDI if the initial specimen that yielded the positive CDT result was collected in an outpatient setting or within the first three calendar days of hospitalization of a patient admitted from home.

Study Population and Data Collection

From September 2014 through December 2015, patients identified through routine surveillance were characterized as having CA-CDI after chart review. All confirmed CA-CDI cases in New Haven County were contacted until a sample of at least 20 cases was reached. Patients were contacted from November 2014 through March 2015 by telephone for an interview. Patients who agreed to participate were screened for the following: an overnight stay in a hospital, nursing home, or long-term care facility in the last twelve weeks; and a prior incidence of CDI. Those who replied yes to any of the screening questions were deemed ineligible and not interviewed further. Patients were then screened for having had any diarrhea (defined as three or more loose stools in a 24-hour period) at the time the stool specimen was collected. Patients who responded yes were deemed eligible to participate and were enrolled in the study as cases after providing consent. Enrolled cases were asked questions regarding demographics, clinical symptoms, health care facility exposure, household members, food and water exposure, antibiotic and other medication use, as well as comorbidities.

After each case was enrolled, a list of landline phone numbers of potential controls was obtained. Potential controls were matched on age bracket (18-29, 30-39, 40-59, 60-69, 70+) and

county. Potential controls who agreed to participate were first screened for the following: an overnight stay in a hospital, nursing home, or long-term care facility in the last twelve weeks; a prior episode of CDI; and diarrhea (defined as three or more loose stools in a 24-hour period) in the twelve weeks prior to the matched case's onset date (referred to as the reference date). Potential controls who responded yes to any of the screening questions were deemed ineligible and not interviewed further. Potential controls who responded no to all the screening questions were deemed eligible and enrolled in the study as controls after providing consent. Controls were asked the same questions as cases for the interview with the exception of clinical symptoms.

Statistical Analysis

An analysis was performed in order to compare the enrolled group of cases with the un-enrolled group of potential cases. The χ^2 test was used to compare categorical demographic characteristics of potential cases and enrolled cases. A χ^2 test was also used to compare categorical demographic characteristics of enrolled cases and enrolled controls.

Conditional logistic regression on matched cases and controls enrolled in the study was performed to test the relationship between each exposure variable and CA-CDI. Odds ratios, 95% confidence intervals, and Wald χ^2 p-values were obtained from the conditional logistic regression analysis. McNemar's test for matched/paired data was performed for comparison with conditional logistic regression. McNemar's matched two-by-two tables were used to calculate odds ratios. Confidence intervals could not be provided by McNemar's test because standard errors are not generated. Conditional logistic regression and McNemar's test have similar null hypotheses but use different models to test them.

Due to the small sample size used in this study, we could not perform a matched analysis on stratified exposures. In order to examine strata defined by whether or not antibiotics were used, an unmatched analysis was used. Two-by-two tables were generated to calculate an unmatched odds ratio, 95% confidence intervals, and χ^2 p-values.

Some variables in the present paper were reformatted to make a binary response variable for two-by-two table analysis. Responses to each of the six food groups asked were initially rated on a 1-4 scale in the interview question (always, sometimes, rarely, never). Responses 1-3 were recoded as 1 and response 4 recoded as 0. A summation of the recoded binary responses to each of the six food groups was calculated to reflect diet diversity. Summed scores between 0 and 4

were again recoded as 0 (not diverse) while summed scores 5-6 were recoded as 1 (diverse). Comorbidity data was categorized into 1 and greater than or equal to 2 to compare two levels of comorbidities. Tap water responses were made into two categories by combining treated and bottled water responses under the assumption that bottled water is also treated.

Individual cases were reviewed for important within two-week exposures to *C. difficile* and the time frame of any antibiotic use relative to their symptom onset date. This data was used to create a timeline to assess the relationship between exposure to *C. difficile*, facilitation of development of symptoms by antibiotics, and symptom onset. A bar chart to visually present this information was also created. Cases who did not report a time frame for antibiotic use were included in the timeline analysis table with an asterisk but left out of the figure.

Microsoft Access Professional Plus 2013 was used for questionnaire forms and data management. All analyses were conducted using statistical software (SAS version 9.4 and SAS OnDemand for Academics) and two-sided $p \leq 0.05$ was considered statistically significant. A p-value of ≤ 0.10 was considered indicating a variable of potential interest for the larger case-control study due to the small sample size of our study (N=42). The figures presenting a timeline of *C. difficile* exposure and CDI symptom development were created in Microsoft Excel 2008 (Version 12.2.0).

RESULTS

Enrollment Process

From November 2014 through March 2015, 74 CA-CDI patients were identified either by *C. difficile* toxin enzyme immunoassay (EIA) or *C. difficile* molecular assay on a stool sample, followed by chart review. Of the 74 patients identified, 50 patients were contacted for interview. Twenty-two patients (44.0%) agreed to participate, were deemed eligible, and enrolled. Twenty-eight patients (56.0%) refused or were unreachable, were ineligible after screening, or were incapacitated. Compared to the 50 potential cases not enrolled, the 22 cases enrolled were not significantly different for the proportion of females (63.6% vs. 68.0%, p=0.81). No significant difference was seen between the not enrolled and enrolled groups for the proportion of patients 70 years and older (43.5% vs. 38.8%, p=0.80).

Enrollee Demographics

Among the 22 cases enrolled, 63.6% were female and 90.9% were white (Table 1). Similarly, of the 20 controls enrolled, 65.0% were female and 85.0% were white. While 18.2% of cases and only 5.3% of controls were Hispanic/Latino(a), this difference was not statistically significant (p=0.35). Cases and controls did not significantly differ for type of health insurance, education level, or income level. Three of 22 cases and 3 of 20 controls were health care workers.

Table 1: Demographic characteristics of cases (N=22) and controls (N=20) in the sample.

	Case N = 22 (%)	Control N = 20 (%)	χ^2 p-value
Ethnicity			0.208
Hispanic/Latino(a)	4 (18.2)	1 (5.0)	
Non-Hispanic/Latino(a)	18 (81.8)	18 (90.0)	
Missing	0	1 (5.0)	
Race			0.563
White	20 (90.9)	17 (85.0)	
Black	2 (9.1)	2 (10.0)	
Other	0 (0.0)	1 (5.0)	
Health Insurance			0.401
Private	7 (31.8)	10 (50.0)	
Public	5 (22.7)	3 (15.0)	
Combination of Private and Public	10 (45.5)	6 (30.0)	
No insurance	0 (0.0)	1 (5.0)	
Education Level			0.934
Some high school	1 (4.6)	1 (5.0)	
High school	4 (18.2)	3 (15.0)	
College/technical school	7 (31.8)	5 (25.0)	
College for four years	10 (45.5)	11 (55.0)	
Income Level			0.991
Less than $15,000	1 (4.5)	1 (5.0)	
Less than $25,000	2 (9.0)	2 (10.0)	
Less than $35,000	1 (4.5)	1 (5.0)	
Less than $50,000	2 (9.0)	1 (5.0)	
Less than $70,000	1 (4.5)	1 (5.0)	
More than $70,000	5 (22.7)	7 (35.0)	
Missing	10 (45.5)	7 (35.0)	
Gender			0.927
Male	8 (36.4)	7 (35.0)	
Female	14 (63.6)	13 (65.0)	
Age Band			0.996
18-29	2 (9.1)	1 (5.0)	
30-39	4 (18.2)	3 (15.0)	
40-49	2 (9.1)	2 (10.0)	
50-59	1 (4.6)	1 (5.0)	
60-69	3 (13.6)	3 (15.0)	
70+	10 (45.5)	10 (50.0)	

Exposure to Possible Risk Factors and Matched Analysis

All potential risk factors for CA-CDI for both cases and controls were assessed during a period of time prior to the matched case's symptom onset date, referred to as the reference date (Table 2). Cases (72.7%) were significantly more likely than controls (15.0%) to have been on an antibiotic in the twelve weeks prior to the reference date (OR=12.00 CI 1.78-512.97, p=0.003, CLR matched analysis; OR=12.00, p=0.002, McNemar's matched analysis). Antibiotics were most likely to be prescribed for a skin or soft tissue infection and dental surgery. There were no significant differences between cases and controls in use of PPI and use of pain, sleep, anti-

smoking, anti-anxiety, or anti-depression medication during the twelve weeks before the reference date (data not shown).

Health care exposures included personal care and/or visit/accompaniment of someone else to a medical facility. For the matched analysis, of the 22 cases enrolled, 20 (90.9%) had a health care exposure sometime in the twelve weeks prior to the reference date. Only 11 (55.0%) of controls had a health care exposure in the same time period. The difference was significant (p=0.016, CLR matched analysis; p=0.008, McNemar's matched analysis) with cases having 9.61 (CI 1.87-undefined) times the odds of a health care exposure compared to controls. Health care exposures were also assessed separately during three time periods: within two weeks, within four weeks, and within twelve weeks of the reference date. Because recent studies have emphasized an incubation period of 2-3 days (McFarland, 1989; Toshniwal, 1981; Samore, 1994), we combined the within four and twelve-week periods to better assess the importance of the within two weeks exposure. Having any health care exposure within two weeks of the reference date was not significantly different for cases and controls, nor was having any health care exposure between two weeks and twelve weeks. Having more than one health care exposure in the twelve weeks prior to the reference date was not significantly different between cases and controls. Being a health care worker/volunteer or having a household member who was a health care worker/volunteer was not significantly different between cases and controls. Thirteen (59.1%) cases and 3 (15.0%) controls had an invasive medical procedure in the twelve weeks prior to the reference date, which was statistically significant (OR=10.00 CI 1.42-433.98, p=0.012, CLR matched analysis; OR=10.00, p=0.007, McNemar's matched analysis).

A greater proportion of cases than controls had other household members, although this was not statistically significant (Table 2). The greatest proportion of both cases and controls had one comorbidity. The difference in having one comorbidity versus no comorbidities, however, was not significantly different between cases and controls. The difference in having at least two comorbidities and no comorbidities was also not significantly different between cases and controls.

Having a diverse diet of microbe-rich foods (scoring a 5 or 6 on the diversity scale) was not significantly different between cases and controls: only 9.1% of cases and no controls ate at least five of the six foods on the list. Cases (72.7%) were significantly more likely to drink

untreated tap water than controls (15.0%) only under McNemar's matched analysis (OR= 3.50, p=0.096).

Table 2: Selected exposure two-by-two tables. Adjusted odds ratios, 95% confidence intervals, and Wald χ^2 p-values generated from a conditional logistic regression model. P-values and odds ratios also calculated from McNemar's Test for matched/paired case-control studies.

Exposure	Case N (%)	Control N (%)	Matched OR (95% CI) (Conditional Logistic Regression)	p-value (Conditional Logistic Regression)	Matched OR (McNemar's test)	p-value (McNemar's test)
Any Medical Care or Visit						
No	2 (9.1)	9 (45.0)	1.00	---	1.00	---
Yes	20 (90.9)	11 (55.0)	9.61 (1.87, undefined)	0.016	undefined	0.008
Multiple Health Care Exposures						
0-1	11 (50.0)	11 (55.0)	1.00	---	1.00	---
2-4	11 (50.0)	9 (45.0)	1.50 (0.17, 17.96)	1.00	2.50	0.655
Any Medical Care/Visit 0-2 Weeks						
No	11 (50.0)	15 (75.0)	1.00	---	1.00	---
Yes	11 (50.0)	5 (15.0)	2.25 (0.63, 10.00)	0.267	2.25	0.166
Any Medical Care/Visit 2-12 Weeks						
No	9 (40.9)	10 (50.0)	1.00	---	1.00	---
Yes	13 (59.1)	10 (50.0)	2.00 (0.29, 22.10)	0.688	2.50	0.414
Any Medical Procedure						
No	9 (40.9)	17 (85.0)	1.00	---	1.00	---
Yes	13 (59.1)	3 (15.0)	10.00 (1.42, 433.98)	0.012	10	0.007
HCW or Household HCW						
No	18 (81.8)	14 (70.0)	1.00	---	1.00	---
Yes	4 (18.2)	6 (30.0)	0.33 (0.01,4.15)	0.625	0.33	0.317
Antibiotic Use						
No	6 (27.3)	17 (85.0)	1.00	---	1.00	---
Yes	16 (72.7)	3 (15.0)	12.00 (1.78, 512.97)	0.003	12.00	0.002
Tap Water						
Treated/Bottled	13 (59.1)	17 (85.0)	1.00	---	1.00	---
Untreated	9 (40.9)	3 (15.0)	3.50 (0.67, 34.53)	0.180	3.50	0.096
Other household members						
No	8 (36.4)	11 (55.0)	1.00	---	1.00	---
Yes	14 (63.6)	9 (45.0)	2.33 (0.53, 13.98)	0.344	2.33	0.206
Comorbidities						
0	4 (18.2))	2 (10.0)	1.00	---	1.00	---
1	17 (77.3)	14 (70.0)	1.50 (0.36, 7.23)	0.754	3.00	0.157
≥ 2	1 (4.5)	4 (20.0)	0.26 (0.00, 1.71)	0.250	0.50	0.414
Diversity of Diet						
0-4	20 (81.8)	20 (75.0)	1.00	---	1.00	---
5-6	2 (9.1)	0 (0.0)	1.00 (0.05, undefined)	1.000	1.67	0.480

Table 3: Stratified, unmatched analysis of exposures, including household diaper use, in Table 2 by antibiotic use. Odds ratios, 95% confidence intervals, and χ^2 p-values generated from a two-by-two table χ^2 test.

Exposure	OR Antibiotics (95% CI)	X^2 test p-value	OR No Antibiotics (95% CI)	X^2 test p-value
Any Medical Care or Visit 0-2 Weeks				
No	1.00	---	1.00	---
Yes	2.00 (0.15, 25.00)	0.596	3.23 (0.46, 25.00)	0.226
Any Medical Care or Visit 2-12 Weeks				
No	1.00	---	1.00	---
Yes	* 0.77 (0.57, 1.03)	0.200	2.86 (20.00, 0.40)	0.283
Any Medical Care or Visit				
No	1.00	---	1.00	---
Yes	* 0.83 (0.68, 1.02)	0.656	5.56 (0.54, 50.00)	0.123
Multiple Health Care Exposures				
0-1	1.00	---	1.00	---
2-4	1.10 (0.08, 14.29)	0.943	1.12 (0.17, 7.14)	0.901
Any Medical Procedure				
No	1.00	---	1.00	---
Yes	3.33 (0.25, 50.00)	0.348	7.69 (0.85, 50.00)	0.051
HCW or Household HCW				
No	1.00	---	1.00	---
Yes	0.67 (0.05, 9.09)	0.764	** 1.49 (1.09, 2.08)	0.133
Tap Water				
Treated/Bottled	1.00	---	1.00	---
Untreated	1.30 (0.96, 1.75)	0.200	4.76 (0.61, 33.33)	0.121
Other household members				
No	1.00	---	1.00	---
Yes	0.50 (0.04, 6.67)	0.596	** 0.54 (0.33, 0.89)	0.013
Comorbidities				
0	1.00	---	1.00	---
1	* 0.83 (0.71, 1.03)	0.517	2.70 (0.26, 33.33)	0.394
≥ 2	0.12 (0.01, 1.72)	0.084	1.20 (0.16, 9.09)	0.858
Diversity of Diet				
0-4	1.00	---	1.00	---
5-6	2.17 (0.14, 33.33)	0.570	1.54 (0.14, 16.67)	0.726
Household Diaper Use* **				
No	1.00	---	1.00	---
Yes	**9.09 (2.44, 33.33)	0.018	16.67 (1.22, 5.88)	0.014

***Relative risk (RR) for cases**

***Relative risk (RR) for controls**

*****Not included in Table 2 because cell values were too small to calculate McNemar's**

Stratification by Antibiotics and Unmatched Analysis

After using an unmatched analysis to stratify by antibiotics, untreated water use was no longer significantly different between cases and controls, for both groups. There was a significant difference between cases and controls who did not take antibiotics and the number of household members (OR=0.54 CI 0.33-0.89, p=0.013, unmatched analysis). Having other household members was protective against CA-CDI in the group that did not take antibiotics.

Cases who did not take antibiotics had a significantly greater odds than controls who did not take antibiotics of having had any medical procedure in the twelve weeks prior to the reference date (OR=7.69 CI 0.85-50.00, p=0.051, unmatched analysis). This relationship was not significantly different for cases and controls who did take antibiotics. Having more than two comorbidities was significantly protective against CA-CDI in the group that did take antibiotics (OR=0.12 CI 0.01-1.72, p=0.084, unmatched analysis). No statistical significance for this relationship was seen in the group that did not take antibiotics. Although household diaper use was not included in the matched analysis in Table 2, in the unmatched analysis stratified by antibiotic use, having a household member who wore diapers in the twelve weeks prior to the reference date was significantly different between cases and controls for both groups. For those who took antibiotics, having a household member who wore diapers was protective against CDI with no cases having had this exposure (RR Controls=9.09 CI 2.44-33.33, p=0.018, unmatched analysis). For those who did not take antibiotics, having a household member who wore diapers was associated with CA-CDI with cases having 16.67 (CI 1.22-5.88) times the odds of having this exposure than controls (p=0.014, unmatched analysis).

As seen in both Table 2 and Table 3, diversity of diet continued to not be significantly different between cases and controls. Also continuing to not be significantly different between cases and controls in the unmatched analysis stratified by antibiotic use were having multiple health care exposures, having any medical care/visit within two weeks of the reference date, and having any medical care/exposure between two and twelve weeks from the reference date.

Timeline Analysis

Table 4 presents a possible timeline for assessing the relationship between *C. difficile* exposure and facilitation of development of CDI symptoms. This table is limited to cases. Exposure events are those in which a case may have come into contact with the bacteria. Facilitating events include certain medications that may allow *C. difficile* to proliferate in the gut such as antibiotics, PPIs, and SSRIs. Of the 22 cases presented in the table, 12 reported a health care exposure within two weeks of their reported symptom onset date. Another 4 cases had an alternative exposure that could potentially account for being exposed to *C. difficile* in the two

weeks prior to symptom onset. These alternative exposures that were considered included the following: case was a health care worker/volunteer; case had a household member who was a health care worker/volunteer; case had a household member who wore diapers; case had a household member who attended daycare. These exposures were considered equivalent to having a health care exposure within two weeks of symptom onset because they are normally on-going exposures that can be assumed to have occurred within two weeks of symptom onset.

Having a household member who had diarrhea and required toilet assistance in the twelve weeks prior to the case's onset date was initially considered as an alternative two-week exposure. However, this was ultimately discarded as an alternative exposure because no time component was included in the interview question. Furthermore, this is typically not an on-going exposure. Consequently, it could not be assumed that the diarrhea occurred within two weeks of the case's symptom onset date.

Of the 22 cases in Table 4, 16 took an antibiotic in the twelve weeks prior to symptom onset date. However, the time frame for this antibiotic use was only recorded for 10 of the cases. The other 6 cases without a time frame for antibiotic use are marked with an asterisk. It was known for these cases that an antibiotic was taken in the twelve weeks prior to symptom onset date, but not whether this antibiotic was taken within two weeks, within four weeks, or within twelve weeks of the symptom onset date.

Figure 1 contrasts the health care or alternative exposure time with facilitating event time for both cases and controls. In Figure 1a, the between two and twelve weeks time frame excludes cases that also had an exposure within two weeks. Other potential *C. difficile* exposures asked about in the interview did not include a time frame question and were not included in the figure. In Figure 1b, the between two and twelve weeks time frame excludes cases that also had a facilitating exposure within two weeks. Other potential CDI facilitating exposure variables collected in the interview did not include a question regarding time frames and were not included in the figure.

Figure 1a shows that a greater number of cases had an exposure, including any health care exposure or alternative exposure, within two weeks than between two and twelve weeks. This pattern is similar for the facilitating exposures (2b), which include antibiotic use, PPI use, and SSRI use. A greater number of cases had a facilitating exposure within two weeks than between two and twelve weeks. Unlike the cases, similar numbers of controls had health care or

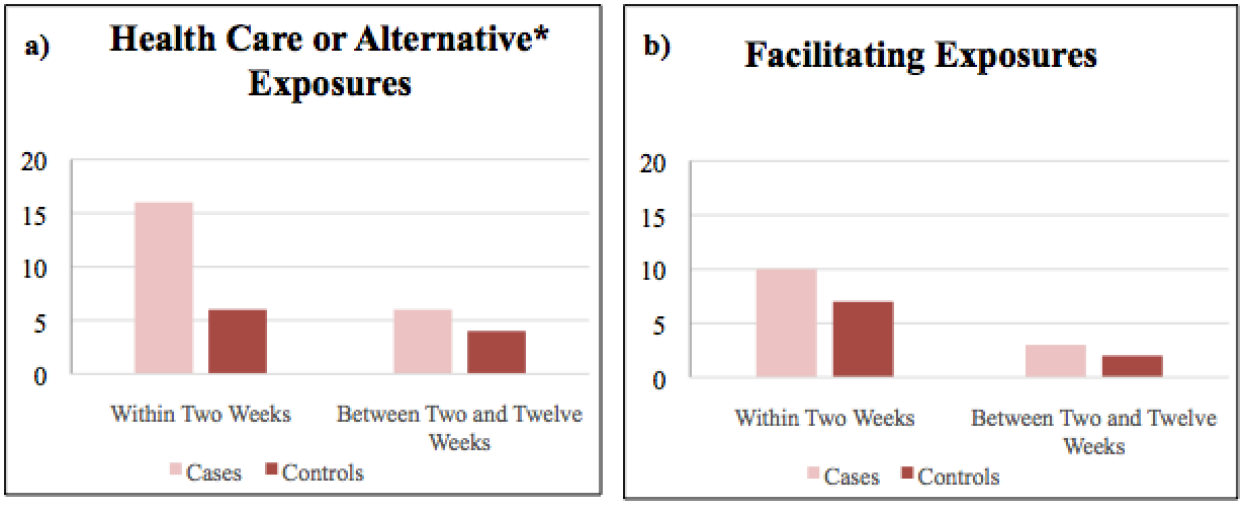
alternative exposures within two weeks of the reference date and between two and twelve weeks of the reference date. Similar numbers of controls also had a facilitating exposure during both time frames.

Table 4: Timeline of two-week health care exposures, alternative two-week exposures, and facilitating exposure for cases.

Case Number	Month of Symptom Onset	Most Recent Health Care Exposure (weeks)	Alternative Exposure	Most Recent Antibiotic Exposure (weeks)	Two-week Potential Exposure to *C. difficile* For
195	Sep	4		4	No
283	Oct	2		2	Yes
284	Oct	4	Household member is a health care worker	*	Yes
348	Oct	2		2	Yes
354	Oct	4	Health care worker	*	Yes
367	Oct	2		---	Yes
371	Oct	2		---	Yes
372	Oct	2		2	Yes
377	Oct	2	Health care worker	*	Yes
399	Nov	2		2	Yes
403	Nov	2		---	Yes
413	Oct	12		*	No
461	Nov	4		*	No
483	Nov	2		2	Yes
509	Nov	4	Health care worker	4	Yes
512	Dec	2		4	Yes
516	Dec	---	---	*	No
526	Dec	2		---	Yes
536	Dec	4		2	No
613	Nov	2		2	Yes
616	Nov	12	Household member with diarrhea, provided toilet assistance	---	No
624	Dec	---	Household member wore diapers and attends daycare	---	Yes

*** Indicates that the case took an antibiotic within twelve weeks of the reference date but was unsure if antibiotic use occurred within two weeks, within four weeks, or within twelve weeks of symptom onset date.**

Figure 1: a) Health care exposures b) Facilitating exposures (antibiotics, proton pump inhibitor, and SSRI use) by case or control status and time from case symptom onset.



***Alternative exposures are those that could have accounted for a case or control being exposed to *C. difficile* within two weeks prior to the symptom onset date. These include: case was a health care worker/volunteer, case had a household member who was a health care worker/volunteer, case had a household member who wore diapers, case had a household member who attended daycare.**

DISCUSSION

Matched Analysis

Our study confirmed the importance of antibiotic use in predicting CDI in a small sample of CA-CDI cases. Other exposures that were significantly different between cases and controls in the matched analysis may not have been independent of antibiotic use. In the matched analysis, we found that in addition to antibiotic use being statistically significant, having had any medical procedure or any medical care/visit in the twelve weeks prior to the reference date were also significantly different between cases and controls. The difficulty in assessing these relationships independently from one another is that antibiotics tend to be prescribed simultaneously with or after a medical care event or procedure. Without stratifying in the matched analysis, we could not say whether or not antibiotic use was a confounding factor for these other exposures. Further analysis must be done in the larger CDC study to assess the interaction between antibiotic use and health care exposures.

Having multiple health care exposures was not significantly different between cases and controls in the matched analysis. This may support the hypothesis that the time at which the health care exposure occurs is more important than the amount of health care exposures that occur in predicting CA-CDI. We therefore continued to assess the timeline of exposure and facilitating event in the rest of the analysis.

Type of drinking water used also proved to be significantly different between cases and controls, with a greater proportion of cases drinking untreated tap water than the corresponding proportion of controls. *C. difficile* bacteria or spores may be more likely to be present in untreated tap water than treated tap water. Antibiotics may kill off the other bacteria in the gut, allow *C. difficile* to proliferate, and cause symptomatic infection.

Unmatched Analysis

The unmatched analysis produced interesting results, which should be examined in the larger CDC study. The unmatched analysis was important in order to control for antibiotic use in order to assess the leading exposures to *C. difficile* for those who did not take antibiotics.

However, the unmatched data allowed for age to be a confounding factor, which had been controlled for in the matched analysis. We were unable to stratify by age bracket in the unmatched analysis because the number of participants with each exposure would have been too small to conduct any interpretable analysis.

The emphasis in the unmatched data was on the group with no antibiotic exposure, since antibiotic use is known to be such a strong predictor of CDI and can facilitate long-standing colonization progressing to disease as well as concurrent exposures. A few exposures were statistically significant among the group that did not take antibiotics, including having other household members, having a household member who wears diapers, and having had any medical procedure in the twelve weeks prior to the reference date. Of particular interest was household diaper use. It has been shown that infants can be colonized with *C. difficile* (Bolton, 1984). This puts family members at risk when coming into contact with infants, especially when changing a diaper. It is conceivable that this exposure could be sufficient to cause symptomatic CA-CDI without antibiotic use in some cases. This explanation may also apply to the significant difference between cases and controls seen for the invasive medical procedure exposure. Some procedures may result in more intensive health care setting exposures to surfaces or hands contaminated with *C. difficile* than others. We suggest that emphasis be placed on these exposures in the larger CDC study to better determine their relationship with CDI.

While having other household members was significantly different between cases and controls, it was shown to be a protective factor against CA-CDI, which was not expected. It is most likely that the sample size was too small to assess a legitimate relationship between other household members and odds of CA-CDI or there was confounding by age. This analysis should be replicated in the larger CDC study. Furthermore, those who took antibiotics and had a household member who wore diapers were protected against CDI. For this result we cannot provide a plausible explanation except for perhaps if there are more controls with young children willing to participate in the study than controls without young children. A similar explanation (if true) could conceivably explain the protective effect shown for at least two comorbidities as compared to no comorbidities in the group that took antibiotics. Perhaps potential controls with more health problems are more willing to further scientific research.

Timeline Analysis

The incubation period for symptomatic CDI following exposure to *C. difficile* is generally thought to be less than two weeks. Most of the cases had a within two weeks exposure to a health care setting or alternative plausible exposure from their date of symptom onset. While our study was unable to confirm an exact incubation period, our results are consistent with those of others (McFarland et al. 1989, and Toshniwal, 1981, and Samore, 1994). Furthermore, most of our cases also had a facilitating exposure that occurred concurrently with or prior to the health care or alternative exposure. It seems that antibiotic use more often comes before exposure to *C. difficile* or at the same time. Perhaps an intervention to prevent CDI would be to discourage those on antibiotics from certain types of high-risk exposures or at least to practice effective hand hygiene when on an antibiotic, especially when coming into contact with a health care facility.

This study confirms the important role antibiotics play in CA-CDI. As for those who do not take an antibiotic and still present with symptomatic CA-CDI, further research must be conducted. Only 6 of the cases in this study did not take an antibiotic in the twelve weeks prior to their symptom onset date. Two of the 6, however, came in direct contact with a household member who either had diarrhea or wore diapers in the twelve weeks prior to their symptom onset date. While the exposures for the other 4 cases are unexplained, these cases provide evidence that antibiotics, health care exposures, and household members with diarrhea and/or diapers increase one's odds of having CA-CDI.

In a case-control study in the United Kingdom, 52% of CA-CDI cases took an antibiotic (Wilcox, 2008). In another study in Denmark, 48% of cases with CA-CDI took an antibiotic (Soes, 2014). Studies conducted in the United States have shown a higher proportion of CA-CDI cases that took an antibiotic (73% Kuntz, 2011; 78% Khanna, 2012). Our study was more consistent with the U.S. proportion of cases with antibiotic use. Because our study was so small, however, we were incapable of assessing a large enough number of cases who lacked an antibiotic exposure in order to determine other risk factors for CA-CDI, particularly exposures that lead directly to CDI without needing facilitation from antibiotics.

Limitations

The greatest limitation in this study was the sample size collected. The small sample size made it difficult to conduct matched analysis without large 95% confidence intervals. It also limited our ability to conduct a stratified analysis and control for age.

Some other limitations existed in the interview and data collection process prior to analysis. First, the interview asked about each exposure followed by a time frame during which the exposure occurred (i.e., within two weeks, within four weeks, within twelve weeks). If the enrollee could not recall the specific time period during which the exposure occurred, the default was to mark the response as within twelve weeks. Consequently, it is possible that the number of within two-week responses is lower than in reality. Had we been given an option for "Unknown time frame," we might have made a stronger argument for the importance of the within two weeks exposure. A similar problem occurred with antibiotic use. As can be seen in Table 4, 6 antibiotic exposure time frames are missing for those who took antibiotics within twelve weeks of the reference date. This is because the interview was structured so that if an enrollee was unable to remember exactly which antibiotic was taken during the twelve weeks prior to the reference date, the time frame question was skipped. It is possible that had the enrollees provided this time frame information, regardless of remembering the name of the antibiotic taken, our evidence for the simultaneous exposure to antibiotics and *C. difficile* could have been stronger.

The final major limitation in our study was the potential for participation bias. It is possible that, as with any case-control study, either cases or controls were differentially more likely to participate than the other if they were somehow invested in the results of the research.

Future Direction for Multistate CDC Study Analysis

While the small sample size used in this study lacked the power to confirm any of the results, our study does present a preview to what may be seen in the analysis of the larger CDC study. We conclude with some suggestions for the CDC to emphasize in their analysis based on our preliminary results.

In the larger study, the risk and protective factors assessed in this study need to be stratified by age bracket. This will help determine whether people of certain ages are more likely

to be exposed to *C. difficile* without a health care exposure and/or without antibiotic use. For example, this could be parents of infants who are wearing diapers, adults living with their elderly parents wearing diapers, or the elderly living with others wearing diapers. A similar potential age bias may exist for cases with household members experiencing diarrhea. These exposures are important to further validate because they may explain part of the changing epidemiology of CDI.

Our study also suggested a strong interaction between health care exposures and antibiotic use. The larger study should assess this interaction to confirm whether or not health care exposures are an important factor in predicting CDI independent of antibiotic use. The larger study should further investigate the significance of the within two weeks health care exposure and the between two and twelve weeks exposure since it is possible our study was not large enough to produce any significant data.

Lastly, we found untreated tap water to be a significant risk factor for CA-CDI cases in Connecticut. It would be interesting to learn if this finding holds up in other EIP sites in the larger CDC study. This finding would open doors for possible interventions to reduce rates of CA-CDI by treating water for *C. difficile*.

Despite the need for further analysis with the larger CDC study to confirm our preliminary results, we feel confident to suggest that a possible intervention for reducing the rate of CA-CDI would be to ensure that those who are on an antibiotic or who have recently taken an antibiotic practice hand hygiene, particularly when in a health care facility.

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