Environmental Contamination in Households of Patients with Recurrent *Clostridium difficile* Infection

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Abstract

Background: Recurrent *Clostridium difficile* infection (R-CDI) is common and difficult to treat, potentially necessitating fecal microbiota transplantation (FMT). Although *C. difficile* spores can persist in the hospital environment and cause infection, little is known about their potential presence in the household environment.

Methods: Households of R-CDI subjects in the peri-FMT period, and of geographically and age-matched controls, were analyzed for presence of *C. difficile*. Household environmental surfaces and fecal samples from humans and pets in the household were examined. Post-FMT subject households were also examined (environmental surfaces only). Participants were surveyed regarding their personal history and household cleaning habits. Environmental and fecal samples were cultured for *C. difficile*. Species identity and molecular characteristics of presumptive *C. difficile* isolates were determined using the PRO kit (Remel, USA), Gram staining, PCR, toxinotyping, *tcdC* gene sequencing, and pulsed-field gel electrophoresis (PFGE).

Results: Environmental cultures detected *C. difficile* on ≥ 1 surface in 8/8 (100%) peri-FMT households vs. 3/8 (38%) post-FMT households and 3/8 (38%) control households (P = 0.025). The most common *C. difficile*-positive surfaces were the vacuum (11/27, 41%), toilet (8/30, 27%), and bathroom sink (5/29, 17%). *C. difficile* was detected in 3/36 (8%) fecal samples (2 R-CDI subjects, 1 household member). Nine (90%) of 10 households with multiple *C. difficile*-positive samples had a single genotype present each.

Conclusions: *C. difficile* was found in the household environment of R-CDI patients. Whether this is a cause or consequence of R-CDI is unknown. If household contamination leads to R-CDI, effective decontamination may be protective.

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Introduction

Infection rates and mortality due to *Clostridium difficile* infection (CDI) are increasing [1-2]. Recurrences of CDI also are common, with 20-30% of patients having a first recurrence, and 45% of these individuals subsequently having a second recurrence [3]. Some patients experience numerous recurrences, which ultimately may necessitate fecal microbial transplantation (FMT). Whether recurrent CDI (R-CDI) is from persistent *C. difficile* gut colonization between episodes, vs. new acquisition of *C. difficile* from the environment, is unknown.

The hospital environment has been extensively studied as an external source for *C. difficile* acquisition. *C. difficile* spores can contaminate the hospital environment of inpatients with CDI, and can persist there for at least 5 months [4], require specific sporicidal cleaning practices (bleach, hydrogen peroxide vapor, UV technology, etc.) for adequate killing [5, 6], and contribute to subsequent transmission and disease [5]. In contrast, little is known regarding the possible presence of *C. difficile* spores in the household environment of CDI patients, including the physical environment and human and animal inhabitants.

Humans and pets can also be asymptomatic *C. difficile* carriers. Reported carriage prevalence rates vary by host group, e.g., 1-3% for healthy adults, 20-30% for recently hospitalized patients, and 51% for long-term care facility residents during a CDI outbreak, consistent with increased carriage as a result of exposure to environmental contamination [7-8]. Up to 70% of healthy newborns and infants are also colonized with *C. difficile* [9]. *C. difficile* colonization has been shown in 10% of healthy household

dogs [10] and up to 40% of cats and dogs at veterinary clinics [11], although whether this relates to CDI in humans is unknown.

The goal of the present study was to define the prevalence, persistence, epidemiologic correlates, and molecular characteristics of *C. difficile* in the household environment of R-CDI subjects, including environmental surfaces, humans, and pets.

Methods

Household enrollment. Subjects ≥ 18 years old with R-CDI who were referred to a University of Minnesota gastroenterology clinic, and were scheduled to undergo FMT in the immediate future, were offered study participation (peri-FMT group). Consideration for FMT required (1) a minimum of 2 spontaneous relapses following the initial CDI episode, each within a month of stopping antimicrobial therapy, and (2) documented failure of an advanced antimicrobial therapy regimen (vancomycin pulse/taper, or vancomycin plus rifaximin chaser). Control subjects of similar age and geographic location to case subject were offered participation as a convenience sample from among the investigators' acquaintances. Co-habiting family members of all ages (defined as sleeping overnight in the same home as the index subject > 50% of the time) were also offered participation. Additionally, subjects from the same gastroenterology clinic who had undergone FMT for R-CDI 6 to 24 months prior to enrollment were offered participation, for household environmental sampling only (post-FMT group). Exclusion criteria for all index subjects included residence in a long-term care or rehabilitation

facility, or relocation to a new home within the previous 30 days. For control subjects, an additional exclusion criterion was a history of CDI or chronic diarrhea. There were no exclusion criteria for household members.

Household visit. Each participating household was visited once by study personnel. Peri-FMT households were visited between 7 days before and 10 days after the FMT procedure. At the beginning of the visit, the index subject and any participating household member(s) gave informed consent for participation. All participating subjects in peri-FMT and control households were given kits to collect a fecal sample, including from any household pets (mammals only).

All participants completed a survey, administered by study personnel, that addressed history of CDI or other diarrheal illnesses, underlying medical conditions, current or recent (within 1 year) antibiotic use or healthcare facility exposure, personal hand hygiene, and CDI knowledge. Additionally, the household member responsible for the greatest share of housecleaning completed a survey regarding the usual cleaning frequency of specific household areas (described below), the estimated date when each area was last cleaned, and whether bleach products were commonly used in each area. A household cleaning frequency value was then calculated for each household, as the mean usual cleaning frequency for each targeted household area. (For additional details regarding the surveys and cleaning frequency scale, see the **Table 1** footnotes.)

Environmental samples were obtained from pre-specified locations. A standard sampling method and list of surfaces was followed according to the "*Clostridium difficile* Household Study Environmental Sample Collection Protocol" from the Centers for Disease Control and Prevention (CDC) (personal communication, Stacy Holzbauer). Environmental samples were obtained with sterile, premoistened sponges (sponge-stick with neutralizing buffer, 3M, USA) using aseptic technique. Surfaces targeted in each household (if applicable) included the inside of the vacuum cleaner bag or intake compartment, diaper changing area, bathroom areas (toilet seat/handle, sinks/faucet, door handles/light switch), kitchen areas (refrigerator handle/shelf, microwave door, kitchen counter used for food preparation/ light switch, sink/faucet), door handles of main exit, telephone, computer keyboard, television remote control, and pet food/water dishes. As a negative control, a sponge was exposed briefly to air and replaced sterilely into the bag. Duplicate samples were permitted; e.g., if a household contained two bathrooms used regularly by the index subject, surfaces in both bathrooms were sampled. Sites sampled in most or all households (i.e., all except the diaper changing area and pet food/water dishes, which were sampled in 1 and 8 households, respectively) were defined as "core environmental sites".

Laboratory evaluation. *C. difficile* isolation from environmental samples was done using a modified CDC protocol. Sterile phosphate buffered saline with 0.1% Tween 80 (50 mL) was added to the sterile bags containing the environmental sample sponges. Bags were placed into a Stomacher 400 circulator (Seward Laboratory Systems, Davie, FL) at 260

RPM for 1 minute. The liquid was removed, placed into sterile centrifuge tubes, and centrifuged at 3500 x g for 15 minutes. Thereafter, 45 mL of buffer was removed and the pellet was resuspended in the remaining buffer. A 0.2 mL aliquot of the resulting suspension was plated in duplicate onto pre-reduced cycloserine-cefoxitin-fructose agar with horse blood and taurocholate (CCFA-HT, Anaerobic systems, USA). Additionally, 1 mL of suspension was inoculated into cycloserine-cefoxitin-fructose broth (CCFB), to help increase the culture yield from the environmental samples [12].

Fecal samples were processed using a single alcohol shock method involving a 1:1 mixture of stool and 95% ethanol. The stool-ethanol mixture was held at room temperature for 45-60 minutes, with brief vortexing every 15 minutes. Samples were centrifuged at 3000 rpm for 10 minutes. After removal of the supernatant, the stool pellet was streaked onto pre-reduced CCFA-HT plates.

All CCFA-HT plates and CCFB broth tubes were incubated at 37ºC under anaerobic conditions for 48-72 hours. Suspected *C. difficile* colonies from CCFA-HT plates were further identified using McLung Toabe agar (criterion: lecithinase and lipase-negative), blood agar (criterion: no hemolysis), PRO kit (Remel, USA), and Gram stain (criterion: spore forming, Gram-positive bacilli). Presumptive *C. difficile* colonies were further characterized by PCR detection of the pathogenicity locus (PaLoc), binary toxin (*cdtB*), and *C. difficile* toxin regulator *tcdC* genes; toxinotyping; and sequence analysis of *tcdC* for specific deletions [13-17]. Molecularly confirmed *C. difficile* isolates also underwent pulsed-field gel electrophoresis (PFGE) analysis, allowing assignment to an

established or novel North American pulsotype (NAP) based on 80% similarity in comparison with CDC reference profiles [18].

Statistical analysis. The three household groups (peri-FMT, post-FMT, and control) were compared according to demographic characteristics and results of environmental and fecal *C. difficile* sampling using Fisher's exact test or student's t-test, with a two-sided $P < 0.05$ considered significant. Number of *C. difficile*-positive core environmental sites per household group was evaluated using Poisson regression to adjust for potential confounding factors, including average case patient age, average number and age of household members, number of pets, household cleaning frequency, and bleach cleaning product use. The likelihood of a particular site yielding *C. difficile* was determined using site-specific logistic regression analysis, with adjustment for usual cleaning frequency and bleach cleaning product use.

The study was approved by the Institutional Review Board at the University of Minnesota.

Results

Household surveillance. Overall, 8 peri-FMT and 8 control households underwent surveillance of the household environment and the human and animal residents.

Additionally, 8 post-FMT households, divided equally between 6 months and 2 years post-FMT, underwent environmental surveillance only. Household characteristics are shown in **Table 1**. The peri-FMT household characteristics are slightly skewed by household #1, which had 5 household members and 3 pets, while most households had between 0-3 household members and 0-2 pets (data not shown). Only 1 control household contained pets, compared with 4 households each in the peri- and post-FMT households. The three groups were otherwise similar according to measured demographic characteristics except for household cleaning frequency (which differed between peri-FMT and post-FMT households) and the index subjects' underlying medical conditions (which differed between peri-FMT and control households).

Environmental samples. All 8 (100%) peri-FMT households, as compared with only 3/8 (38%) control households, had at least one *C. difficile*-positive environmental sample (P = 0.025) (**Table 2**). Likewise, with 31 (10%) of 326 total core environmental samples positive for *C. difficile*, the mean number of *C. difficile*-positive core environmental samples was significantly greater for peri-FMT households (2.6) than control households (0.25) (P = 0.024) (**Table 3**). Among post-FMT households, the likelihood of *C. difficile* environmental contamination decreased with increasing elapsed time since FMT, with 2/4 (50%) 6-months-post-FMT households but only 1/4 (25%) 2-years-post-FMT households having any *C. difficile*-positive surface (P > 0.1). Post-FMT households had a mean of only 1 *C. difficile*-positive core environmental sample per household, which did not differ significantly from control households (**Table 3**). The number of *C. difficile*-

positive core environmental sites per household (as estimated using Poisson regression) for a given household group did not shift appreciably when adjusted for mean age of the index patient, mean number and age of household members, number of pets, household cleaning frequency, and bleach cleaning product use (**Table 3**).

Although the 31 *C. difficile*-positive core environmental samples (i.e., all but one *C. difficile*-positive diaper changing area sample from a control household) were from diverse household sites, certain high-prevalence sites were over-represented (**Table 4**). The site most likely to be *C. difficile*-positive was the vacuum (11/27 [41%]), followed by the toilet (8/30 [27%]), and bathroom sink/faucet (5/29 [17%]). According to separate site-specific logistic regression models for each of the 3 most commonly contaminated sites (vacuum, toilet, bathroom sink/faucet), the odds of detecting *C. difficile* were not affected by usual cleaning frequency or bleach cleaning product use on the site.

Fecal samples. Overall, of 36 fecal samples submitted, 3 (8%) yielded *C. difficile* (**Table 2**). All 16 index subjects from peri-FMT and control households provided a fecal sample; of these, two index subjects from peri-FMT households (vs. none from control households) were *C. difficile*-positive. Both subjects had undergone FMT within 1 week prior to sample collection, and ultimately failed the FMT procedure due to R-CDI (vs. none of 6 *C. difficile*-negative peri-FMT index subjects; P = .036). As for other household members, participation rates were high, with only 1 peri-FMT household and 2 control households lacking fecal samples from household members. No participating household members reported a history of CDI or chronic diarrhea. Among the 12

sampled household members, the only *C. difficile*-colonized subject was from a peri-FMT household. None of the 8 pet fecal samples from peri-FMT households were *C. difficile*-positive. (No pet fecal samples were submitted from the only control household with pets.)

Molecular characterization. Table 5 shows the by-household number of environmental samples obtained, number and distribution of *C. difficile*-positive sites, and corresponding pulsotype. Nine (90%) of 10 households that yielded ≥ 2 *C. difficile* isolates had a single *C. difficile* NAP type each, with the sole exception, peri-FMT household #5, having 2 different *C. difficile* NAP types (**Figure 1)**. Anecdotally, peri-FMT household #5, which had the highest proportion of *C. difficile*-positive surfaces (8/15, 53%), was the only household to report significant fecal contamination of the household environment during a R-CDI episode. Control household # 4, which had a *C. difficile*-positive vacuum cleaner, consisted of a single female whose now deceased husband had CDI 6 years prior to the study visit. Although impossible to confirm, we speculate that the *C. difficile* isolate found in the vacuum cleaner may have been present since the husband's illness.

Table 6 shows the molecular characteristics of the 35 *C. difficile* isolates. Two (6%) isolates were unavailable for molecular analysis, since archived stocks were nonviable. Of the 33 available isolates, all were toxin-producing, with the exception of the isolate from the diaper changing area of a young child in control household #2, which was a non-toxigenic strain (consistent with the high prevalence of asymptomatic *C.*

difficile colonization in young children). The most common molecular characteristics overall included NAP6 (11/33, 33%), toxinotype 0 (23/32, 72%), no binary toxin (25/33, 76%), and no *tcdC* deletion (25/32, 78%). The epidemic BI/NAP1/027 *C. difficile* strain [1-2] was uncommon, occurring only in peri-FMT household # 4. NAP7 is the strain most commonly found in animals, however neither of the 2 households which contained NAP7 included pets.

Discussion

This pilot household survey involving R-CDI patients requiring FMT and community controls yielded findings that support three main conclusions. First, peri-FMT households had a significantly higher prevalence of *C. difficile* contamination (100%) than did post-FMT and control households (38% each), and significantly more *C. difficile*-positive sites per household, which usually all yielded the same *C. difficile* genotype. Second, specific household sites (vacuum cleaner and bathroom areas) were relatively high-risk for *C. difficile* contamination. Third, *C. difficile* persisted in the household environment, as evidenced by findings in post-FMT households, although the positivity rate did appear to decrease as more time elapsed. These novel findings have potential clinical relevance, since whereas the hospital environment has received abundant attention in relation to CDI acquisition, household contamination has not yet been studied, and the increasing burden of R-CDI among non-hospitalized patients calls for closer attention to the household environment as a potential source for recurrence.

The significantly greater prevalence and extent of *C. difficile* contamination in peri-FMT households, as compared with post-FMT and control households, could mean that having a household member with CDI leads to household contamination, and/or that household contamination predisposes to R-CDI unless the CDI patient undergoes FMT to re-establish the normal gut microbiota. The possibility that the contaminated household environment may contribute to R-CDI has important practical implications that call for further study of this topic. Most *C. difficile*-positive households had between 1-3 positive environmental sites each. Peri-FMT household #5 was a notable exception, with over 50% of environmental sites contaminated, which anecdotally might be explained by the significant fecal contamination of the household environment that reportedly occurred during the case patients' most recent R-CDI episode. Overt fecal contamination of the household environment was not reported by any other household. Whether fecal incontinence is a risk factor for R-CDI due to more extensive environmental contamination warrants further study.

Among the 10 households with ≥ 2 *C. difficile*-positive samples, all isolates from a given household represented the same PFGE NAP type, with the exception of peri-FMT household # 5, which had 2 separate NAP types. Some CDI patients develop R-CDI due to a different *C. difficile* strain than caused the initial episode, especially with long durations between R-CDI episodes [19-22]. Additionally, fecal samples from a single CDI episode may contain multiple *C. difficile* strains [23]. Either or both of these phenomena could explain the finding of multiple *C. difficile* types within a patient and their household.

The vacuum cleaner was the most common *C. difficile*-positive site. This likely reflects some combination of its function as an aggregator (by sampling extensive surface areas within the home), the difficulty with using potentially damaging sporicidal agents to clean carpeted floors/rugs, and the infeasibility of cleaning the vacuum cleaners interior. Whether *C. difficile*-contaminated vacuum cleaners constitute a reservoir, potentially leading to transmission/acquisition (via airborne dissemination or direct contact) and new CDI episodes, is unknown.

Not surprisingly, bathroom sites were also frequently contaminated. Most households reported regular bathroom cleanings, including with bleach. Peri-FMT households had the highest reported cleaning frequency and rates of bleach use, likely reflecting prior knowledge regarding *C. difficile* transmission and anxiety induced by their extensive R-CDI history. Although this robust cleaning history seemingly conflicts with these households' high *C. difficile* prevalence, the household cleaning survey addressed only cleaning frequency and bleach use, not specifics such as which particular areas within a given site were cleaned (e.g., whether toilet cleaning included the handle, seat, and/or bowl), whether the bleach product was fresh, how long bleach dwelled before being removed, etc. Therefore, sites conceivably were not cleaned thoroughly/properly, which could allow *C. difficile* to persist despite regular bleach cleaning.

In this regard, hospital-based studies have shown that suboptimal cleaning techniques are insufficient to kill *C. difficile* spores, with 7/9 (78%) *C. difficile*-contaminated hospital rooms remaining *C. difficile*-positive after routine terminal bleach cleaning by hospital housekeeping staff, as compared with only 1/9 (11%) after intensive

bleach cleaning by dedicated research staff [24]. A reason commonly cited for the low overall success of routine hospital room decontamination is the wide variability in cleaning techniques [25]. A study evaluating contamination of healthcare workers' hands also suggested that daily focused cleaning actually may be required to decrease hospital room contamination [26]. Similar considerations may apply in the household, with insufficient cleaning thoroughness or frequency possibly allowing persistent surface contamination despite use of bleach products. A goal for future research would be identify effective and affordable approaches to household *C. difficile* contamination, and to study them in relation to acquisition of *C. difficile* and R-CDI risk.

This study has several limitations. First, the cross-sectional, point-prevalence design may have missed transient *C. difficile* colonization of people, pets, and/or the environment that nonetheless might contribute to recurrence. For instance, *C. difficile* colonization in healthy dogs has been shown to be very transient [10]. Second, the small number of participating households limits statistical power. Third, since this was a pilot prevalence survey, no effort was made to quantify the amount of *C. difficile* present, which conceivably could vary in relation to study group or any of the other epidemiological variables, and might influence transmission risk. Fourth, previous and current *C. difficile* fecal isolates were lacking from most peri-FMT patients for comparison with their household environmental isolates, since specimens were unavailable from the patients' prior CDI episodes and many subjects had extensive recent oral vancomycin use, so unsurprisingly were *C. difficile*-negative when sampled. Fifth, poor recall and/or incomplete honesty may have reduced survey validity. Sixth, most

households reported recent household cleaning, despite our request for no extra cleaning prior to the study visit. Although we nonetheless found frequent *C. difficile* contamination, even more samples conceivably might have been positive without the extra cleaning. Lastly, since the FMT households included only extreme cases of R-CDI, the results may not be broadly applicable, e.g., to patients after a first CDI episode.

At present, whether *C. difficile* environmental contamination is a cause or simply a consequence of R-CDI is unknown. Future research should include longitudinal household surveillance for changes in the prevalence, density, and distribution of *C. difficile* contamination, in relation to R-CDI. If persistence of spores in the household environment leads to recolonization and/or reinfection of patients, and effective methods of household *C. difficile* decontamination can be identified, intensified household cleaning approaches, akin to those already used in hospitals, conceivably could lead to reduced R-CDI rates.

Table 1. Household demographics of subjects with recurrent *Clostridium difficile* infection (R-CDI), peri-fecal microbiota transplantation (FMT) and post-FMT, and subjects in control households.

Demographics	Peri-FMT (n = 8)	Post-FMT (n = 8)	Control (n = 8)
Age (years), mean ± SD	69 ± 14	70 ± 14	68 ± 7
No. female (%)	5 (62)	6 (75)	8 (100)
Time (days) since FMT, mean ± SD, (median) [†]	-25 ± 78[a, †] (2)	443 ± 280[b, †] (399)	N/A
R-CDI duration (mo.)[c], mean ± SD	10.1 ± 8.3	7.6 ± 3.7	N/A
No. of CDI episodes**,** mean ± SD	3.6 ± 1.0	4.3 ± 1.3	N/A
CDI requiring hospitalization, no. (%)	3 (38)	5 (67)	N/A
Healthcare facility exposure in past 6 mo.[d], no. (%)	8 (100)	6 (75)	6 (75)
Antibiotic use in past 12 mo.[e], no. (%)	5 (63)	5 (63)	1 (13)
Acid reducing medication[f] use, no. (%)	1 (13)	5 (63)	1 (13)
Diarrhea at time of study visit, no. (%)	3 (38)	2 (25)	1 (13)
No. household members (mean, range)	10 (1.25, 0-5)	6 (0.75, 0-3)	7 (0.88, 0-3)
Age (years) of household members, mean ± SD	59 ± 15	58 ± 21	57 ± 24
No. pets (mean per household)	8 (0.8)	6 (0.75)	2 (0.25)
Household cleaning frequency,[g] mean ± SD[†]	4 ± 0.6[†]	3.2 ± 0.5[†]	3.4 ± 0.7
No. bleach cleaning (%)	6 (75)	6 (75)	4 (50)

Hand washing,[h] mean ± SD	4.8 ± 0.7	5 ± 0	4.9 ± 0.4
CDI knowledge,[i] mean ± SD	6.8 ± 1.3	6.6 ± 2.4	7.9 ± 1.9
No. with index subject with ≥ 2 underlying medical conditions[g] (%)[‡]	5 (62) [‡]	4 (50)	0 (0) [‡]

[†]P < 0.05 for comparison of peri-FMT to post-FMT households using student's t-test (2-sided).

[‡]P < 0.05 for comparison of peri-FMT to control households using Fisher's exact test (2-sided).

[a]Mean time since FMT is 2.4 ± 5.6 excluding outlier of – 217 days (FMT delayed unexpectedly after study visit due to chemotherapy).

[b]Mean ± SD for 6 months post-FMT group is 197 ± 36, mean ± SD for 2 years post-FMT group is 689 ± 143.

[c]Duration is prior to FMT or study visit (in case of subject whose FMT was delayed unexpectedly for 217 days) for peri-FMT group, prior to FMT for post-FMT group.

[d]Exposure is defined as > 1 hour spent in a hospital, emergency room/urgent care, outpatient clinic, hemodialysis unit, or long term care facility.

[e]Antibiotic use did not include antibiotics used for CDI therapy.

[f]Proton pump inhibitors and/or H2 receptor blockers.

[g]Household cleaning frequency is mean of cleaning frequency for each environmental surface sampled in the household, with 5 = < 1/week, 4 = 1/week, 3 = every other week, 2 = 1/month, 1 = < 1/month, 0 = never.

[h]5 = always, 4 = most of the time, 3 = some of the time, 2 = rarely, 1 = never.

[i]Number of questions correct out of 9 total questions regarding CDI risk factors.

[j]Checklist of underlying conditions assessed included diabetes, lung disease, heart disease (including hypertension), liver disease, cancer, rheumatologic disease, inflammatory bowel disease, recipient of bone marrow or solid organ transplant.

Table 2. Isolation of *Clostridium difficile* from environmental and fecal samples obtained during household surveillance in relation to fecal microbiota transplantation (FMT).

		Proportion positive for *C. difficile* (%)			
Household group (no. households)	Households[a]	Environmental samples	Case patients	Household members	Pets
Peri-FMT (8)	8/8 (100)[b]	21/106 (19)[c]	2/8 (25)	1/9 (11)	0/8 (0)
Post-FMT (8)					
6 mo. (4)	2/4 (50)	7/57 (12)[d]	N/A[e]	N/A[e]	N/A[e]
2 yrs. (4)	1/4 (25)	1/54 (2)	N/A[e]	N/A[e]	N/A[e]
Control (8)	3/8 (38)[b]	3/109 (3)[c,d]	0/8 (0)	0/3 (0)	0/0 (0)

[a]Indicates ≥ 1 sample (environmental or fecal) in the household positive for *C. difficile*.

[b]P = 0.025, [c]P = 0.001, [d]P = 0.03 compared to control (Fisher's exact test, two tailed).

[e]N/A, not applicable; fecal samples were not collected from post-FMT households.

Table 3. Number of core[a] environmental samples positive for *Clostridium difficile* according to household group membership and fecal microbiota transplantation (FMT) status.

Household group	Number of core environmental samples *C. difficile*-positive, mean ± SE						
	Unadjusted	Adjusted[b] based on:					
		Household members, mean no.	Case patient, mean age	Household members, mean age	Pets, mean no.	Household cleaning frequency	Bleach cleaning
Peri-FMT	2.63 ± 0.75	2.60 ± 0.55	2.62 ± 0.63	1.72 ± 0.21	2.75 ± 0.74	2.80 ± 0.99	2.67 ± 0.76
Post-FMT	1.00 ± 0.53	0.86 ± 0.45	0.99 ± 0.55	0.62 ± 0.47	1.00 ± 0.55	0.95 ± 0.53	1.02 ± 0.53
Control	0.25 ± 0.15	0.23 ± 0.14	0.31 ± 0.18	0.16 ± 0.14	0.22 ± 0.14	0.24 ± 0.15	0.24 ± 0.15

[a] Sites sampled in most or all households.

[b]Adjustment was done using Poisson regression with robust covariance estimators.

Table 4. Origin of 31 *Clostridium difficile*-positive core[a] environmental samples from surveillance of 24 peri-fecal microbiota transplant (FMT), post-FMT, and control households.

Environmental site	No. samples[b]	*C. difficile*-positive, no. (% total samples)	Usual cleaning frequency, OR (95% CI)[c]	Bleach cleaning, OR (95% CI)[c]
Vacuum cleaner	27[b]	11 (41)	1.8 (0.98 – 3.4)	--
Peri-FMT	9	6 (67)	--	--
Post-FMT	9	3 (33)	--	--
Control	9	2 (22)	--	--
Toilet	30[b]	8 (27)	3.37 (0.9 – 13.1)	0.55 (0.08 – 3.9)
Peri-FMT	10	7 (70)	--	--
Post-FMT	11	1 (9)	--	--
Bathroom sink/faucet	29[b]	5 (17)	N/A[d]	0.60 (0.05 – 6.8)
Peri-FMT	9	4 (44)	--	--
Post-FMT	10	1 (10)	--	--
Computer	24[b]	2 (8)	N/A[e]	N/A[e]

Peri-FMT	7	1 (14)	--	--
Post-FMT	9	1 (11)	--	--
Bathroom door/light switch	27[b]	1 (4)	N/A[e]	N/A[e]
Post-FMT	9	1 (11)	--	--
Microwave	24[b]	1 (4)	N/A[e]	N/A[e]
Peri-FMT	8	1 (13)	--	--
Refrigerator	24[b]	1 (4)	N/A[e]	N/A[e]
Peri-FMT	8	1 (13)	--	--
Remote	24[b]	1 (4)	N/A[e]	N/A[e]
Peri-FMT	7	1 (14)	--	--
Telephone	24[b]	1 (4)	N/A[e]	N/A[e]
Post-FMT	8	1 (13)	--	--

[a]Core sites excluded pet food/water dishes and diaper changing areas, since these were not tested for most households (pet foot/water dishes = 8 households, diaper changing area = 1 household).

[b]Includes total number of samples obtained from that site for all 24 households surveyed. Number may be > 24 due to duplicate sites sampled in a household (e.g., 2 separate toilets in 1 household). Results per individual household group may not sum to total as negative household groups not shown.

[c]Site-specific logistic regression, analyzed by using samples collected at each site for all household groups total, not per individual household group.

[d]Unable to determine, as all households with a *Clostridium difficile*-positive bathroom sink reported the same cleaning frequency.

[e]Unable to determine due to low number of *Clostridium difficile*-positive sites.

Table 5. Individual household surveillance results of *Clostridium difficile*-positive environmental (environ.) sites, including pulsed-field gel electrophoresis (PFGE) type.

Household group	Household number	No. of environ. samples	*C. difficile*-positive environ. samples (row %)	*C. difficile*-positive sites	PFGE type
Peri-FMT	1[a]	15	1 (7)	Vacuum	NAP10
	2	14	2 (14)	Toilet, vacuum	NAP2
	3	13	3 (23)	Toilet, bathroom sink, vacuum	NAP7
	4	12	2 (17)	Remote, vacuum	NAP1
	5	15	8 (53)	Microwave, toilet #2	NAP7
				Refrigerator, computer, toilet # 1, bathroom sink #1 and #2, vacuum	NAP6
	6[b]	14	2 (14)	Toilet, bathroom sink	NAP6
	7[b]	13	1 (8)	Toilet	NAP4
	8	16	2 (13)	Toilet, vacuum	NAP6
Post-FMT	1	16	0 (0)	--	

	2	13	4 (31)	Bathroom sink, bathroom door, telephone, vacuum	NAP11
	3	14	0 (0)	--	
	4	16	3 (19)	Computer, vacuum #1	NAP11
				Vacuum #2	ND[c]
	5	13	0 (0)	--	
	6	14	1 (7)	Toilet	Unnamed[d]
	7	15	0 (0)	--	
	8	13	0 (0)	--	
Control	1	15	0 (0)	--	
	2	14	1 (7)	Diaper changing area[e]	Unnamed[d]
	3	13	0 (0)	--	
	4	13	1 (8)	Vacuum	ND[c]
	5	13	0 (0)	--	
	6	13	0 (0)	--	
	7	14	1 (7)	Vacuum	NAP4

	8	16	0 (0)	--

[a]Household member *C. difficile* positive.

[b]Case patient *C. difficile* positive.

[C]ND, not done: archived stocks did not regrow for PFGE analysis despite extensive efforts.

[d]Unnamed (CD isolate profile was not ≥ 80% similar to a Center for Disease Control and Preventions NAP standard).

[e]Non-toxigenic strain (PCR negative for *tcdC* gene and toxinotyping (toxins A and B), and positive for pathogenicity locus gene).

Table 6. Molecular characteristics of 35 *Clostridium difficile* isolates (32 environmental, 3 human).

Household	Source	Binary toxin (*cdtB)*	Toxino-type	*tcdC* deletion	PFGE[a] type
Peri-FMT 1	Vacuum	Neg.	0	0 bp[b]	NAP10
Peri-FMT 1	Household member	Neg.	0	0 bp	NAP10
Peri-FMT 2	Toilet	Neg.	0	0 bp	NAP2
Peri-FMT 2	Vacuum	Neg.	0	0 bp	NAP2
Peri-FMT 3	Toilet	Pos.	V	39 bp	NAP7
Peri-FMT 3	Bathroom sink	Pos.	V	39 bp	NAP7
Peri-FMT 3	Vacuum	Pos.	V	39 bp	NAP7
Peri-FMT 4	Remote	Pos.	III	18 bp	NAP1
Peri-FMT 4	Vacuum	Pos.	III	18 bp	NAP1
Peri-FMT 5	Microwave	Pos.	V	39 bp	NAP7
Peri-FMT 5	Refrigerator	Neg.	0	0 bp	NAP6
Peri-FMT 5	Toilet #1	Neg.	0	0 bp	NAP6
Peri-FMT 5	Bathroom sink #1	Neg.	0	0 bp	NAP6
Peri-FMT 5	Computer	Neg.	0	0 bp	NAP6
Peri-FMT 5	Toilet #2	Pos.	V	39 bp	NAP7
Peri-FMT 5	Bathroom sink #2	Neg.	0	0 bp	NAP6
Peri-FMT 5	Vacuum	Neg.	0	0 bp	NAP6
Peri-FMT 6	Toilet	Neg.	0	0 bp	NAP6
Peri-FMT 6	Bathroom sink	Neg.	0	0 bp	NAP6
Peri-FMT 6	Case patient	Neg.	0	0 bp	NAP6

Peri-FMT 7	Toilet	Neg.	N/A[c]	0 bp	NAP4
Peri-FMT 7	Case patient	Neg.	N/A[c]	0 bp	NAP4
Peri-FMT 8	Toilet	Neg.	0	0 bp	NAP6
Peri-FMT 8	Vacuum	Neg.	0	0 bp	NAP6
Post-FMT 2	Bathroom sink	Neg.	0	0 bp	NAP11
Post-FMT 2	Bathroom door	Neg.	0	0 bp	NAP11
Post-FMT 2	Telephone	Neg.	0	0 bp	NAP11
Post-FMT 2	Vacuum	Neg.	0	0 bp	NAP11
Post-FMT 4	Computer	Neg.	0	0 bp	NAP11
Post-FMT 4	Vacuum 1	Neg.	0	0 bp	NAP11
Post-FMT 4	Vacuum #2	N/A[d]	N/A[d]	N/A[d]	N/A[d]
Post-FMT 6	Toilet	Neg.	0	0 bp	Unnamed[e]
Control 3	Diaper changing area	Neg.	N/A[f]	N/A[f]	Unnamed[e]
Control 6	Vacuum	N/A[d]	N/A[d]	N/A[d]	N/A[d]
Control 9	Vacuum	Neg.	0	0 bp	NAP4

[a]Pulsed-field gel electrophoresis.

[b]bp = base pair.

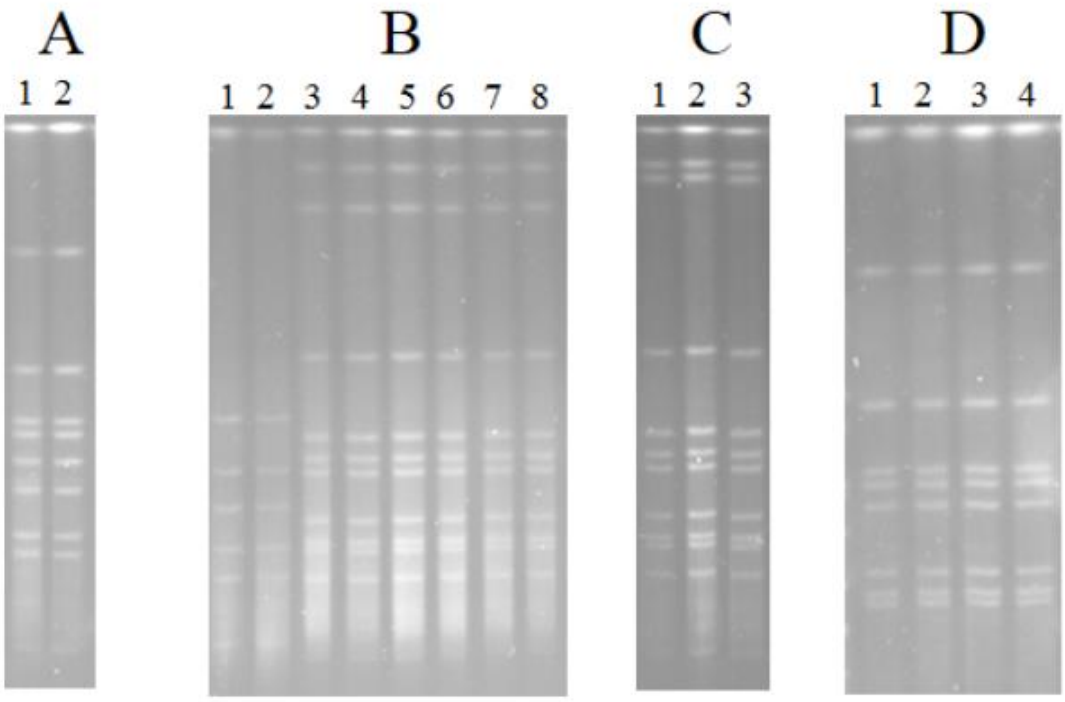
[c]Unable to distinguish between toxinotypes XVIII and XXIX due to identical appearance of A3 fragment between the two toxinotypes.

[d]Unable to complete molecular characterization since archived stocks did not regrow.

[e]Unnamed (household *C. difficile* isolate profile was not ≥ 80% similar to a defined Center for Disease Control and Preventions NAP standard).

[f]Non-toxigenic strain, so toxins A, B and *tcdC* genes not present.

Figure 1. Pulsed-field gel electrophoresis results of a representative selection of *Clostridium difficile* isolates obtained from household surveillance. (A) Peri-fecal microbiota transplantation (FMT) household #4, with indistinguishable NAP1 isolates in lanes 1 (remote control) and 2 (vacuum). (B) Peri-FMT household #5, with indistinguishable NAP7 isolates in lanes 1 (microwave door) and 2 (toilet #2), and indistinguishable NAP6 isolates in lanes 3 (refrigerator), 4 (toilet #1), 5 (bathroom sink #1), 6 (computer), 7 (bathroom sink #2), and 8 (vacuum). (C) Peri-FMT household #6, with indistinguishable NAP6 isolates in lanes 1 (toilet), 2 (bathroom sink), and 3 (case patient fecal sample). (D) Post-FMT household #2, with indistinguishable NAP11 isolates in lanes 1 (bathroom sink), 2 (bathroom door), 3 (telephone), and 4 (vacuum).



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