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

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Recurrent *Clostridium difficile* infection (R-CDI) is common and difficult to treat, potentially necessitating fecal microbiota transplantation (FMT). Although *C. difficile* spores persist in the hospital environment and cause infection, little is known about their potential presence or importance in the household environment. Households of R-CDI subjects in the peri-FMT period and of geographically matched and age-matched controls were analyzed for the presence of *C. difficile*. Household environmental surfaces and fecal samples from humans and pets in the household were examined. Households of post-FMT subjects were also examined (environmental surfaces only). Participants were surveyed regarding their personal history and household cleaning habits. Species identity and molecular characteristics of presumptive *C. difficile* isolates from environmental and fecal samples were determined by using the Pro kit (Remel, USA), Gram staining, PCR, toxinotyping, *tcdC* gene sequencing, and pulsed-field gel electrophoresis (PFGE). Environmental cultures detected *C. difficile* on ≥1 surface in 8/8 (100%) peri-FMT households, versus 3/8 (38%) post-FMT households and 3/8 (38%) control households ($P = 0.025$). The most common *C. difficile*-positive sites 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 (two R-CDI subjects and one household member). Nine (90%) of 10 households with multiple *C. difficile*-positive samples had a single genotype present each. In conclusion, *C. difficile* was found in the household environment of R-CDI patients, but whether it was found as a cause or consequence of R-CDI is unknown. If household contamination leads to R-CDI, effective decontamination may be protective.

Infection rates and mortality due to *Clostridium difficile* infection (CDI) are increasing (1, 2). Recurrence of CDI is also common, with 20 to 35% 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 lead to fecal microbiota transplantation (FMT). Whether recurrent CDI (R-CDI) is from persistent *C. difficile* gut colonization between episodes, versus new acquisition of *C. difficile* from the environment, is unknown.

The hospital environment has been studied extensively as an external source of *C. difficile* acquisition. *C. difficile* spores contaminate the hospital environment of inpatients with CDI and can persist there for at least 5 months (4), requiring sporicidal cleaning practices (bleach, hydrogen peroxide vapor, and UV technology, etc.) for adequate killing (5, 6) and contributing 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. One Canadian study of households in which no individual had a CDI history found *C. difficile* in 5.3% of sites in 31% of households, with ribotype 027 being the most common *C. difficile* variant (25% of isolates) (7).

Humans and pets can carry *C. difficile* asymptomatically. Reported carriage prevalence rates vary by group, e.g., 6 to 13% for healthy adults, 20 to 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 and increased exposure to antimicrobials in hospitals and long-term-care facilities (8–11). Up to 70% of healthy newborns and infants are also colonized with *C. difficile* (12). *C. difficile* colonization has been shown in 10% of healthy household dogs (7) and in up to 40% of cats and dogs at veterinary clinics (13), although whether this relates to CDI transmission to humans is unknown. In a household study of dogs, in the few instances in which a household yielded both canine and environmental *C. difficile* isolates, the isolates exhibited different ribotypes, although most *C. difficile* isolates represented toxigenic strains previously reported in humans (7).

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

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MATERIALS AND 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 (i) a minimum of two spontaneous recurrences following the initial CDI episode, each within a month of stopping of antimicrobial therapy, and (ii) documented recurrence after an extended antimicrobial therapy regimen (vancomycin pulse/taper or vancomycin plus a rifaximin chaser). The FMT donor for all patients was an unrelated, "universal" donor prescreened for *C. difficile* and other potential pathogens.

Control subjects with ages and geographic locations similar to those of the case subjects were recruited for participation. Control subjects did not work in the health care setting and were either one degree removed from the investigator (i.e., were previously unknown to the investigator) or an acquaintance of a study team member who had no direct contact with CDI patients and/or *C. difficile* laboratory isolates.

Cohabiting 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. Study personnel visited each participating household once. To accommodate logistical issues regarding scheduling of home visits, peri-FMT homes 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 fecal samples 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 health care 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 frequency of cleaning 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 frequency of cleaning of each targeted household area. (For additional details regarding the surveys and cleaning frequency scale, see Table 1.)

Environmental samples were obtained from prespecified locations. A standard sampling method and standard list of surfaces were followed according to a household surveillance protocol from the Centers for Disease Control and Prevention (CDC) (Stacy Holzbauer, personal communication). Environmental samples were obtained with sterile, premoistened sponges (sponge stick with neutralizing buffer; 3M, USA) by using an aseptic technique. Surfaces targeted in each household (if applicable) included the inside area of the vacuum cleaner bag or intake compartment (to potentially provide a general assessment of household contamination); diaper changing areas; bathroom areas (toilet seat/handle, sinks/faucet, and door handles/light switch); kitchen areas (refrigerator handle/shelf, microwave door, kitchen counter used for food preparation/light switch, and sink/faucet); pet food/water dishes; and high-touch areas such as door handles of the main exit, telephones (including cell phones), computer keyboards, and television remote controls. The area sampled was not calculated for each surface given the wide variety of both two- and three-dimensional surfaces sampled. However, a uniform sampling ap-

TABLE 1 Household demographics of subjects receiving FMT for recurrent CDI and index control subjects

Demographic	Value for group		
	Peri-FMT ($n = 8$)	Post-FMT ($n = 8$)	Control[d] ($n = 8$)
Mean age (yr) ± SD	69 ± 14	70 ± 14	68 ± 7
No. (%) of females	5 (62)	6 (75)	8 (100)
Mean time (days) since FMT ± SD (median)	2.4 ± 5.6[a,c] (2)	443 ± 280[b,c] (399)	NA
Mean duration of recurrent CDI (mo)[e] ± SD	10.1 ± 8.3	7.6 ± 3.7	NA
Mean no. of CDI episodes ± SD	3.6 ± 1.0	4.3 ± 1.3	NA
No. (%) of CDI patients requiring hospitalization	3 (38)	5 (67)	NA
No. (%) of patients with healthcare exposure in past 6 mo[f]	8 (100)	6 (75)	6 (75)
No. (%) of patients with antibiotic use in past 12 mo[g]	5 (63)	5 (63)	1 (13)
No. (%) of patients using acid-reducing medication[h]	1 (13)	5 (63)	1 (13)
No. (%) of patients with diarrhea at time of study visit	3 (38)	2 (25)	1 (13)
Total no. of household members (mean no. of household members per household group, range)	10 (1.25, 0–5)	6 (0.75, 0–3)	7 (0.88, 0–3)
Mean age (yr) of household members ± SD	59 ± 15	58 ± 21	57 ± 24
No. of pets (mean no. of pets per household)	8 (0.8)	6 (0.75)	2 (0.25)
Mean household cleaning frequency score[i] ± SD[c]	4 ± 0.6[c]	3.2 ± 0.5[c]	3.4 ± 0.7
No. (%) of patients reporting bleach cleaning	6 (75)	6 (75)	4 (50)
Mean hand washing score[j] ± SD	4.8 ± 0.7	5 ± 0	4.9 ± 0.4
Mean CDI knowledge score[k] ± SD	6.8 ± 1.3	6.6 ± 2.4	7.9 ± 1.9
No. (%) of index subjects with ≥2 underlying medical conditions[l]	5 (62)[m]	4 (50)	0 (0)[m]

[a] The mean time since FMT as shown excludes a single outlier case (−217 days) for whom FMT was delayed unexpectedly after the study visit due to chemotherapy. Including this case, the mean time since FMT ± the standard deviation is −25 ± 78 days.

[b] The mean ± standard deviation for the post-FMT group at 6 months is 197 ± 36 days; the mean ± standard deviation for the post-FMT group at 2 years is 689 ± 143 days.

[c] $P < 0.05$ for comparison of peri-FMT to post-FMT households using Student's *t* test (two sided).

[d] NA, not applicable; no CDI or FMT in control subjects.

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

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

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

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

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

[j] A score of 5 is always, 4 is most of the time, 3 is some of the time, 2 is rarely, and 1 is never.

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

[l] Underlying conditions assessed included diabetes, lung disease, heart disease (including hypertension), liver disease, cancer, rheumatologic disease, inflammatory bowel disease, and stem cell or solid-organ transplantation.

[m] $P < 0.05$ for comparison of peri-FMT to control households (by two-sided Fisher's exact test).

proach was taken for each household surface regardless of the specific size/shape; i.e., the same part of the toilet (handle and top/bottom/sides of seat) was sampled in each household. As a negative control, a sponge was exposed briefly to air and re-placed 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, which accounted for nearly all samples, were defined as "core environmental sites."

Culture methods. *C. difficile* isolation from environmental samples was done by using a CDC protocol (Stacy Holzbauer, personal communication). The CDC methods were not modified significantly for our study, to allow for a potential future comparison of our results with those of CDC-led household studies. A 50-ml aliquot of sterile phosphate-buffered saline with 0.1% Tween 80 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 min. The liquid was removed, placed into sterile centrifuge tubes, and centrifuged at 3,500 × *g* for 15 min. 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 prereduced cycloserine-cefoxitin-fructose agar with horse blood and taurocholate (CCFA-HT; Anaerobe Systems, Morgan Hill, CA). Additionally, 1 ml of the suspension was inoculated into prereduced cycloserine-cefoxitin-fructose broth (CCFB), which also included 0.1% sodium taurocholate, to help increase the culture yield from the environmental samples (14).

Fecal samples were processed by 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 to 60 min, with brief vortexing every 15 min. Samples were centrifuged at 3,000 rpm for 10 min. After removal of the supernatant, the stool pellet was streaked onto prereduced CCFA-HT plates.

All CCFA-HT plates and CCFB tubes were incubated at 37°C under anaerobic conditions for 48 to 72 h. Suspected *C. difficile* colonies from CCFA-HT plates were further identified by using McLung Toabe agar (criterion, lecithinase and lipase negative), blood agar (criterion, no hemolysis), the Pro kit (Remel, USA), and Gram staining (criterion, spore-forming, Gram-positive bacilli). Isolates fulfilling all these phenotypic criteria were regarded as *C. difficile*. Although plates may have contained >1 *C. difficile* colony, a single colony was picked arbitrarily for further molecular characterization.

Molecular analysis. The selected *C. difficile* colonies were characterized molecularly as performed routinely by the Minnesota Department of Health Laboratory within its ongoing CDC-sponsored *C. difficile* surveillance projects (Stacy Holzbauer, personal communication). Briefly, DNA was extracted from cultures grown on plates of trypticase soy agar (TSA II; Becton Dickinson) plus 5% sheep blood agar under anaerobic conditions by using an InstaGene matrix (Bio-Rad, CA) according to the manufacturer's protocol. Isolated DNA samples underwent PCR to determine the presence/absence of the pathogenicity locus (PaLoc) (to differentiate between toxigenic and nontoxigenic strains), the presence/absence of the binary toxin gene (*cdtB*), and toxinotyping (based on restriction site variation within the PaLoc) (15–19). Additionally, the *C. difficile* toxin regulator gene *tcdC* was amplified and partially sequenced by using two previously reported primers, forward primer C2 (17) and reverse primer tcdc1 (20). This partial gene sequencing provided the characterization of the first 470 bp of the gene, targeting previously noted *tcdC* polymorphisms plus various deletions of 1, 18, 36, or 39 bp, some of which resulted in premature stop codons and a theoretically truncated protein (21). The resulting partial *tcdC* sequences were compared to partial and complete *tcdC* gene sequences available at the PubMLST database (http://pubmlst.org/cdifficile/) and were labeled accordingly. Any study sequences that matched multiple entries in the PubMLST *tcdC* allele database (which differed only with respect to polymorphisms outside the region of *tcdC* sequenced here) were labeled with the multiple corresponding PubMLST alleles. Pulsed-field gel electrophoresis (PFGE) analysis of *C. difficile* isolates was performed according to CDC protocols, allowing assignment to an established North American pulsotype (NAP) based on ≥80% similarity to one of the CDC's 12 defined NAP reference profiles (20).

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 by using Fisher's exact test or Student's *t* test, with a two-sided *P* value of <0.05 being considered significant. The number of *C. difficile*-positive core environmental sites per household group was evaluated by using Poisson regression to adjust for potential confounding factors. A basic model adjusted only for the number of samples collected per household; an enhanced model also adjusted for average index subject age, average number and age of household members, number of pets, and bleach cleaning product use. The likelihood of a particular site yielding *C. difficile* was determined by using site-specific logistic regression analysis, with adjustment for usual cleaning frequency and the use of a bleach cleaning product. 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. The three groups were similar according to measured demographic characteristics (Table 1), 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. The numbers of environmental samples collected per household (overall median, 13; range, 11 to 15) were similar across groups (not shown). All 8 (100%) peri-FMT households, 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 environmental samples positive for *C. difficile*, according to Poisson regression analysis, the number of *C. difficile*-positive environmental samples per household was significantly higher for peri-FMT households than for control households (Table 2). This was true with both adjustment for only the number of samples per household ($P = 0.007$) (Table 2) and additional adjustment for the number and age of household members, number of pets, number of individuals with *C. difficile*, and use of bleach cleaning products ($P = 0.04$). The post-FMT households had results that were bracketed by those of the peri-FMT and control households for both the proportion of households with at least one *C. difficile*-positive sample and the proportion of positive samples (Table 2). However, the post-FMT households' results appeared to diverge in relation to time since FMT, with the households at 6 months post-FMT ($n = 4$) having numerically higher values that resembled more closely those of the peri-FMT households and with the households at 2 years post-FMT ($n = 4$) having numerically lower values that resembled more closely those of the control households (Table 2).

Although the 31 *C. difficile*-positive core environmental samples were from diverse household sites, certain high-prevalence sites were overrepresented (Table 3), such as the vacuum cleaner (11/27 samples [41%]), 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

TABLE 2 Isolation of *Clostridium difficile* from environmental and fecal samples obtained during household surveillance in relation to FMT

Household group (no. of households)	No. of *C. difficile*-positive samples/total no. of samples (% positive samples; 95% CI)[a]				
	Households[b]	Environmental samples	Index subjects	Household members	Pets
Peri-FMT (8)	8/8 (100; 68–100)[c]	21/106 (19; 6–23)[d,e]	2/8 (25; 7–59)	1/9 (11; 0.6–44)	0/8 (0; 0–22)[f]
Post-FMT (8)	3/8 (38; 14–69)[c]	8/111 (7; 4–14)[e,g]	NA	NA	NA
6 mo (4)	2/4 (50; 15–85)	7/57 (12; 6–23)	NA	NA	NA
2 yr (4)	1/4 (25; 13–70)	1/54 (2; 0.1–10)	NA	NA	NA
Control (8)	3/8 (38; 14–69)[c]	3/109 (3; 0.9–8)[d,g]	0/8 (0; 0–32)	0/3 (0; 0–56)	NA

[a] CI, confidence interval; NA, not applicable (fecal samples were not collected from post-FMT households or from pets in control households).
[b] Counted as positive if ≥1 core environmental sample from the household yielded *C. difficile*.
[c] $P = 0.026$ for peri-FMT versus post-FMT or versus the control (by two-tailed Fisher's exact test).
[d] $P = 0.006$ for peri-FMT versus the control (by Poisson regression analysis, with adjustment for the number of samples per household).
[e] $P = 0.016$ for peri-FMT versus post-FMT (by Poisson regression analysis, with adjustment for the number of samples per household).
[f] Pets included 5 dogs and 3 cats.
[g] The *P* value was nonsignificant for post-FMT versus the control (by Poisson regression analysis, with adjustment for the number of samples per household).

TABLE 3 Origins of 31 *Clostridium difficile*-positive core[a] environmental samples from surveillance of 24 peri-FMT, post-FMT, and control households

Environmental site and household type	No. of samples[b]	No. of *C. difficile*-positive samples (% of total samples)
Vacuum cleaner	27[b]	11 (41)
Peri-FMT	9	6 (67)
Post-FMT	9	3 (33)
Control	9	2 (22)
Toilet	30[b]	8 (27)
Peri-FMT	10	7 (70)
Post-FMT	11	1 (9)
Bathroom sink/faucet	29[b]	5 (17)
Peri-FMT	9	4 (44)
Post-FMT	10	1 (10)
Computer	24[b]	2 (8)
Peri-FMT	7	1 (14)
Post-FMT	9	1 (11)
Bathroom door/light switch	27[b]	1 (4)
Post-FMT	9	1 (11)
Microwave	24[b]	1 (4)
Peri-FMT	8	1 (13)
Refrigerator	24[b]	1 (4)
Peri-FMT	8	1 (13)
Remote control	24[b]	1 (4)
Peri-FMT	7	1 (14)
Telephone	24[b]	1 (4)
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 food/water dishes in 8 households and diaper changing area in 1 household).
[b] Includes the total number of samples obtained from the indicated site for all 24 households surveyed and for all 8 households in each group with any positive sample. The number may be >24 (all households) or >8 (individual household groups) due to duplicate sites being sampled per household (e.g., 2 separate toilets in 1 household). The number of samples per individual household group may not sum to the total number, since household groups without positive samples for a given site are not shown separately.

sites (vacuum, toilet, and bathroom sink/faucet), the odds of detecting *C. difficile* were not affected by usual cleaning frequency or use of a bleach cleaning product on the site (data not shown).

Fecal samples. Overall, of the 36 total fecal samples submitted from humans and animals, 3 (8%) yielded *C. difficile* (Table 2). All 16 index subjects from peri-FMT and control households provided a fecal sample, 2 of which (both from subjects of peri-FMT households 6 to 7 days after the FMT procedure) were *C. difficile* positive. Four of the eight peri-FMT household index subjects had their fecal samples collected prior to the FMT procedure and were receiving oral vancomycin at the time of their study visit. No other index subjects in any household groups were receiving anti-*C. difficile* antimicrobials at the time of the study visit. As for other household members, participation rates were high: only 1 peri-FMT household and 2 control households had a household member who did not provide fecal a sample. No participating household members reported a history of CDI or chronic diarrhea. Among the 12 sampled household members, the 1 subject (8%) who was colonized by *C. difficile* 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 single control household with pets.)

Molecular characterization. Table S1 in the supplemental material shows the number of environmental samples obtained, the number and distribution of *C. difficile*-positive sites, and the corresponding PFGE types by household. Notably, 9 (90%) of 10 households that yielded ≥2 *C. difficile* isolates had a single *C. difficile* PFGE type each. The sole exception, peri-FMT household 5, had 2 different *C. difficile* PFGE types.

Table 4 shows the full molecular characteristics of the 35 *C. difficile* isolates (1 colony per positive sample), excluding 2 (6%) isolates that were unavailable for molecular analysis due to nonviable archived stocks. All but 1 of the 33 available isolates were presumptively toxigenic, the sole exception being an isolate from the diaper changing area of a young child in control household 3. The observed molecular variation included four toxinotypes (plus null), four *tcdC* deletion patterns (plus null), five *tcdC* alleles (plus null), and eight pulsotypes. For each characteristic, the most common variant was composed of pulsotype NAP6 (11/33; 33%), toxinotype 0 (23/32; 72%), no binary toxin (25/33; 76%), no *tcdC* deletion (25/32; 78%), and *tcdC* allele fragment 7 (corresponding to complete gene allele 19) (23/33; 70%). The pulsotype was the

TABLE 4 Molecular characteristics of 35 *Clostridium difficile* isolates (32 environmental and 3 human isolates)

Household	Source	Presence of binary toxin (*cdtB*)	Toxinotype	*tcdC* deletion(s) (bp)	*tcdC* allele fragment(s) (full gene allele)[a]	PFGE type
Peri-FMT 1	Vacuum	Negative	0	0	3 (19), 22	NAP10
	Household member	Negative	0	0	3 (19), 22	NAP10
Peri-FMT 2	Toilet	Negative	0	0	2 (3)	NAP2
	Vacuum	Negative	0	0	2 (3)	NAP2
Peri-FMT 3	Toilet	Positive	V	39	5 (20)[b]	NAP7
	Bathroom sink	Positive	V	39	5 (20)[b]	NAP7
	Vacuum	Positive	V	39	5 (20)[b]	NAP7
Peri-FMT 4	Remote	Positive	III	1 and 18	1 (1 or 55)[c]	NAP1
	Vacuum	Positive	III	1 and 18	1 (1 or 55)[c]	NAP1
Peri-FMT 5	Microwave	Positive	V	39	5 (20)[b]	NAP7
	Refrigerator	Negative	0	0	3 (19), 22	NAP6
	Toilet 1	Negative	0	0	3 (19), 22	NAP6
	Bathroom sink 1	Negative	0	0	3 (19), 22	NAP6
	Computer	Negative	0	0	3 (19), 22	NAP6
	Toilet 2	Positive	V	39	5 (20)[b]	NAP7
	Bathroom sink 2	Negative	0	0	3 (19), 22	NAP6
	Vacuum	Negative	0	0	3 (19), 22	NAP6
Peri-FMT 6	Toilet	Negative	0	0	3 (19), 22	NAP6
	Bathroom sink	Negative	0	0	3 (19), 22	NAP6
	Case patient	Negative	0	0	3 (19), 22	NAP6
Peri-FMT 7	Toilet	Negative	XVIII/XXIX[d]	0	3 (19), 22	NAP4
	Case patient	Negative	XVIII/XXIX[d]	0	3 (19), 22	NAP4
Peri-FMT 8	Toilet	Negative	0	0	3 (19), 22	NAP6
	Vacuum	Negative	0	0	3 (19), 22	NAP6
Post-FMT 2	Bathroom sink	Negative	0	0	3 (19), 22	NAP11
	Bathroom door	Negative	0	0	3 (19), 22	NAP11
	Telephone	Negative	0	0	3 (19), 22	NAP11
	Vacuum	Negative	0	0	3 (19), 22	NAP11
Post-FMT 4	Computer	Negative	0	0	3 (19), 22	NAP11
	Vacuum 1	Negative	0	0	3 (19), 22	NAP11
	Vacuum 2	NA[e]	NA[e]	NA[e]	NA[e]	NA[e]
Post-FMT 6	Toilet	Negative	0	0	4	Und[f]
Control 3	Diaper changing area	Negative	NA[g]	NA[g]	NA[g]	Und[f]
Control 6	Vacuum	NA[e]	NA[e]	NA[e]	NA[e]	NA[e]
Control 9	Vacuum	Negative	0	0	3 (19), 22	NAP4

[a] *tcdC* allele designations are based on the taxonomy shown in the PubMLST database (http://pubmlst.org/cdifficile/) for the gene fragment and the complete gene (in parentheses). Fragment 22 has no corresponding complete gene sequence in the database. Fragment 1 corresponds to complete gene alleles 1 and 55, for which the differentiating polymorphisms are outside the region of the fragment.

[b] *tcdC* nonsense mutation C184T results in a premature stop codon, resulting in a truncation of the TcdC protein from 232 amino acids (wild type) to a predicted 61-amino-acid product (19).

[c] Single nucleotide deletion at base 117 of *tcdC* results in a frameshift and premature stop codon, resulting in a predicted 65-amino-acid product (19).

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

[e] Unavailable for molecular characterization (archived stocks did not regrow).

[f] Und, undefined (i.e., the household *C. difficile* isolate's profile was <80% similar to a defined Centers for Disease Control and Prevention NAP standard). These two profiles were also <80% similar to one another.

[g] Nontoxigenic strain; PCR negative for *tcdC* and the toxinotyping PCR targets (which include *tcdA* and *tcdB*) but positive for a 769-bp product by pathogenicity locus PCR, as seen in nontoxigenic strains (as opposed to the ~19-kb product predicted for toxigenic strains).

most discriminating characteristic; all other characteristics were homogeneous within a given pulsotype. The epidemic BI/NAP1/ribotype 027 *C. difficile* strain (1, 2) was uncommon, occurring only in peri-FMT household 4.

DISCUSSION

This survey of households of R-CDI patients who were treated with FMT and community controls yielded findings that support two main conclusions. First, peri-FMT households had a significantly higher prevalence of *C. difficile* contamination (100%) than did control or post-FMT households (38% each) and had significantly more *C. difficile*-positive sites per household than did control households, which, within a given household (with a single exception), all yielded the same *C. difficile* genotype (based on the typing of a single colony from environmental sites). Second, specific household sites (vacuum cleaner and bathroom areas) were at a relatively high risk for *C. difficile* contamination. 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 nonhospitalized patients calls for closer attention to the household environment as a potential source for recurrence.

The greater prevalence and extent of *C. difficile* contamination in peri-FMT households than in control and post-FMT households could mean that having a household member with R-CDI leads to household contamination and/or that household contamination predisposes one to R-CDI unless the CDI patient undergoes FMT to reestablish the normal gut microbiota. The possibility that the contaminated household environment may contribute to R-CDI has potentially important clinical implications that call for further study of this topic.

Of the typing methods used, PFGE was the most discriminating and corresponded with data obtained by using other molecular characteristics, although the results may have been limited by the fact that only one colony from each environmental site was typed. In our study, PFGE types usually differed by household. In contrast, among the 10 households with ≥2 *C. difficile*-positive samples, all isolates from a given household represented the same PFGE type, except for peri-FMT household 5, with its 2 PFGE types. The association of the "multiple-positives" phenomenon with peri-FMT households, and the fact that all negative-control samples were culture negative, suggests that the multiple-positives phenomenon represents extensive within-household contamination with *C. difficile* rather than an artifact, e.g., from cross-contamination during sample collection. It is known that some CDI patients develop R-CDI due to a different *C. difficile* strain than the one that caused the initial episode, especially with long durations between R-CDI episodes (22–25). Additionally, fecal samples from a single CDI episode may contain multiple *C. difficile* strains (26). 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 subjects' possible reluctance to use potentially damaging sporicidal agents to clean carpeted floors/rugs, and the infeasibility of cleaning the interior of a vacuum cleaner. Whether *C. difficile*-contaminated vacuum cleaners constitute a reservoir, potentially leading to transmission/acquisition (e.g., via airborne dissemination or direct contact), is unknown.

Not surprisingly, bathroom sites were also frequently contaminated. Most households reported regular bathroom cleanings, including cleanings with bleach products. Although this cleaning history seemingly conflicts with some of the households' high *C. difficile* prevalence, this may be confounded by recall bias. Also, the household cleaning survey addressed only cleaning frequency and bleach use but not specifics such as which particular areas within a given site were cleaned (e.g., whether toilet cleaning included the flush handle, seat, and/or bowl), whether the bleach product was fresh, and how long bleach was allowed to dwell before being removed, etc. Therefore, sites conceivably were not cleaned optimally, 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 housekeeping staff, compared with only 1/9 (11%) after intensive bleach cleaning by dedicated research staff (27). A reason commonly cited for the low overall success of routine hospital room decontamination is the wide variability in cleaning techniques (28). Similar considerations may apply in the household, with insufficient cleaning thoroughness or frequency possibly allowing surface contamination to persist despite the use of bleach products.

This study has a number of limitations. First, the small number of households constrained statistical power. Second, the cross-sectional, point prevalence design may have missed transient *C. difficile* contamination or colonization. For example, *C. difficile* colonization of healthy dogs can be very short-lived (12). Third, control subjects had connections (albeit indirect) to the investigators and had fewer underlying medical conditions and somewhat higher CDI knowledge scores than did FMT recipients. Fourth, in this exploratory prevalence survey, we did not quantify the *C. difficile* burden in environmental samples; this conceivably could vary with epidemiological variables and might influence transmission risk but would be highly resource-intensive to assess. Fifth, molecular characterization of only one *C. difficile* colony per sample may have missed the presence of multiple *C. difficile* strains. Sixth, most FMT patients lacked *C. difficile* fecal isolates, precluding comparisons with environmental isolates. Seventh, incomplete recall and/or honesty may have reduced survey validity. Eighth, most households reported having cleaned prior to being sampled, despite our request that they not do so, which possibly reduced the positivity rate. Finally, our inclusion of only extreme R-CDI cases may restrict generalizability.

At present, whether *C. difficile* environmental contamination is a cause or 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. Although determination of whether persistent environmental spores lead to R-CDI would be difficult, this hypothesis would be supported if environmental persistence is documented while the case patient transitions from negative to positive fecal samples, followed by clinical recurrence. If persistence of spores in the household environment leads to recolonization of patients, and effective methods of household *C. difficile*

decontamination can be identified, then appropriate household cleaning may potentially reduce R-CDI rates.

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