

Original Article

Increased environmental sample area and recovery of *Clostridium difficile* spores from hospital surfaces by quantitative PCR and enrichment culture

Kevin Antoine Brown PhD^{1,4}, Laura K. MacDougall PhD¹, Kim Valenta PhD⁷, Andrew Simor MD^{2,3}, Jennie Johnstone MD, PhD^{1,3}, Samira Mubareka MD, PhD^{2,3}, George Broukhanski PhD¹, Gary Garber MD^{1,3,5}, Allison McGeer MD^{3,6} and Nick Daneman MD, MSc^{1,2,3}

¹Public Health Ontario, Toronto, Canada, ²Sunnybrook Health Sciences Center, Toronto, Canada, ³Department of Medicine, University of Toronto, Toronto, Canada, ⁴Dalla Lana School of Public Health, University of Toronto, Toronto, Canada, ⁵Department of Medicine, University of Ottawa, Ottawa, Canada, ⁶Mt Sinai Healthcare System, Toronto, Canada and ⁷Faculty of Environmental Sciences, McGill University, Toronto, Canada

Abstract

Objective: *Clostridium difficile* spores play an important role in transmission and can survive in the environment for several months. Optimal methods for measuring environmental *C. difficile* are unknown. We sought to determine whether increased sample surface area improved detection of *C. difficile* from environmental samples.

Setting: Samples were collected from 12 patient rooms in a tertiary-care hospital in Toronto, Canada.

Methods: Samples represented small surface-area and large surface-area floor and bedrail pairs from single-bed rooms of patients with low (without prior antibiotics), medium (with prior antibiotics), and high (*C. difficile* infected) shedding risk. Presence of *C. difficile* in samples was measured using quantitative polymerase chain reaction (qPCR) with targets on the 16S rRNA and toxin B genes and using enrichment culture. **Results:** Of the 48 samples, 64.6% were positive by 16S qPCR (geometric mean, 13.8 spores); 39.6% were positive by toxin B qPCR (geometric mean, 1.9 spores); and 43.8% were positive by enrichment culture. By 16S qPCR, each 10-fold increase in sample surface area yielded 6.6 times (95% CI, 3.2–13) more spores. Floor surfaces yielded 27 times (95% CI, 4.9–181) more spores than bedrails, and rooms of *C. difficile*-positive patients yielded 11 times (95% CI, 0.55–164) more spores than those of patients without prior antibiotics. Toxin B qPCR and enrichment culture returned analogous findings.

Conclusions: *Clostridium difficile* spores were identified in most floor and bedrail samples, and increased surface area improved detection. Future research aiming to understand the role of environmental *C. difficile* in transmission should prefer samples with large surface areas.

(Received 24 January 2018; accepted 8 April 2018)

Clostridium difficile is a gram-positive, anaerobic, spore-forming bacterium; it is a leading cause of healthcare-associated infective diarrhea. Risk factors for the disease include previous hospitalizations, advanced age, and the use of antibiotics.^{1–3} *Clostridium difficile* spores play an important role in disease transmission. Spores can persist in the environment for several months and are resistant to stresses such as heat, oxygen, and disinfectants.^{4,5} *Clostridium difficile* spores are thought to be ubiquitous in the hospital environment,⁶ and contaminated hospital surfaces may serve as an important reservoir for transmission.⁷

However, optimal and standardized sampling and laboratory methods for assessing density of environmental *C. difficile* are not

known. Increasing surface area may maximize the potential to capture spores and push *C. difficile* quantities over the detection limit of a given laboratory method, enabling more precise measurements,^{8,9} but it might also increase the concentration of inhibitors and reduce detection. Larger surface areas also require larger sampling devices, and sponges are preferred over swabs for sampling such areas due to their larger size.^{10,11}

Other challenges include how to consistently measure average environmental burden across surface types (eg, bedrails, floors, and other surfaces), and whether culture or culture-independent methods yield more reliable quantitative measures of the density of environmental *C. difficile*. Bedrails represent one of the most frequently touched surfaces in the hospital environment.¹² Floors, while not a high-touch environment, are often the most *C. difficile*-contaminated surfaces in hospitals; thus, they may play a role in transmission.¹³ While culture techniques may be most readily linked to potential transmission and infection, culture-independent techniques may nevertheless improve quantification of the existing spore burden and risk.⁸

Author for correspondence: Kevin Antoine Brown, Public Health Ontario, 480 University Ave, #300, Toronto, ON M5G1V2, Canada. E-mail: kevin.brown@oahpp.ca or kevin.brown@utoronto.ca

Cite this article: Brown KA, et al. (2018). Increased environmental sample area and recovery of *Clostridium difficile* spores from hospital surfaces by quantitative PCR and enrichment culture. *Infection Control & Hospital Epidemiology* 2018, 39, 917–923. doi:10.1017/ice.2018.103

In this study, we sought to identify whether increased sample surface area was associated with increased detection of *C. difficile* from environmental samples collected from patient rooms in a tertiary-care hospital in a nonoutbreak setting. We explored whether this relationship existed for both floor and bedrail samples, and across several different microbiologic methods, including qPCR and enrichment culture.

Methods

Design and setting

We used an efficient split-plot sampling design^{14,15} to assess the impact of sample surface area on *C. difficile* detection from hospital environmental samples while controlling for room-level clustering of bacterial burden. Small surface-area and large surface-area pairs of bedrail and floor environmental samples were selected from 12 rooms (N=48) in 3 inpatient units (2 general medicine, 1 intensive care), over the course of 2 days in September 2017 in a large tertiary-care hospital located in Toronto, Canada.

Sample collection

For sampling, patient rooms were selected based on a categorization of risk of contamination: high-risk rooms were defined as those of patients with active *C. difficile* diarrhea identified by hospital infection control staff; medium-risk rooms were those of patients with a history of in-hospital antibiotic use in the last 14 days; and low-risk rooms were those of patients without a history of *C. difficile* or in-hospital antibiotic use in the previous 14 days. All high-risk rooms available in the hospital were selected (N=5), as well as a selection of medium-risk (N=2) and low-risk rooms (N=5) on the same wards. All samples were collected independently of ward- and room-cleaning schedules, and the timing of most recent cleaning was not known or recorded. The hospital environmental cleaning policy specified daily routine cleaning and terminal cleaning as surface cleaning with quaternary ammonium. *Clostridium difficile* cleaning was surface cleaning with accelerated hydrogen peroxide performed twice daily.¹⁶

In each selected room, 2 pairs of samples were collected using sterile cellulose sponges premoistened in Dey-Engley neutralizing buffer (Scigene, Scarborough, ON). The first pair consisted of a small surface-area floor sample (32 × 32 cm, 0.10 m²) and a large surface-area floor sample (100 × 100 cm, 1 m²). These samples were collected from contiguous spaces alongside the patient bed on the side of usual access. The second pair consisted of a small surface-area bedrail sample (7.7 × 30 cm, 0.023 m²) and a large surface-area bedrail sample (77 × 30 cm, 0.23 m²). These samples extended down the side of the bedrail panel. Again, these samples were contiguous and from the bedrail on the side of usual access. Small surface-area samples were taken from the portion of the bedrail corresponding to the head of the bed, while large surface-area samples included the remainder of the bedrail. Surfaces were wiped using perpendicular, overlapping “S” patterns using a defined protocol.¹⁷

Outcome measurements

Clostridium difficile cells were extracted from sponge swabs. Briefly, 40 mL sterile double-distilled water was added to the sample bag, which was then vigorously massaged between fingers for 90 seconds. Extracted cells and environmental debris were

collected from the liquid by centrifugation at 7,500 × g for 15 minutes. All but 1 mL of the supernatant was removed, and the pellet was resuspended and transferred to a microcentrifuge tube, which was centrifuged at 10,000 × g for 5 minutes. The entire supernatant was removed, and the pellet was resuspended in 500 µL of 20% Dey-Engley neutralizing broth (Scigene) diluted in sterile water.

With half of the sample, DNA was extracted using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Irvine, CA) using a 1-hour bead-beating step and a DNA elution volume of 50 µL. We conducted quantitative polymerase chain reaction (qPCR) on 2 targets. A 157-bp conserved region of the *C. difficile* 16S rRNA gene (present in ~10 copies per genome) and an internal TaqMan probe (Applied Biosystems, Foster City, CA) were used for detection of all *C. difficile* strains.¹⁸ A 127-bp region of the toxin B gene (present in 1 copy per genome) and a corresponding TaqMan probe were used to specifically detect toxigenic *C. difficile* strains.¹⁹ Reactions (10 µL) containing 5 µL JumpStart Taq ReadyMix for qPCR (D7440, Sigma-Aldrich, St Louis, MO), 0.1 µL reference dye for qPCR (R4526, Sigma), 0.4 µL 25mM MgCl₂, 0.5 µM (each) forward and reverse primers, 0.15 µM TaqMan probe, and 3.85 µL DNA were prepared. These were run in triplicate on an ABI 7900HT thermocycler (Applied Biosystems) under the following conditions: 50°C for 2 minutes, 95°C for 10 minutes, 45 cycles at 95°C for 15 seconds, 60°C for 1 minute. The other half of the sample was enriched and cultured using banana broth (Hardy Diagnostics, Santa Maria, CA).¹⁹ Samples positive by enrichment culture were ribotyped (Appendix 1).

We coded 5 outcome variables. First, 16S qPCR positivity and toxin B qPCR positivity were coded such that any threshold cycle (Ct) < 45 was considered positive, and any Ct ≥ 45 (or undetermined) was considered negative. The enrichment cultured-based measure was coded as either positive or negative. Second, we converted qPCR Ct to spore counts for 16S qPCR and for toxin B qPCR based on their respective standard curves. These *C. difficile* standard curves were generated by serial dilution of a spore stock quantitated using a hemocytometer. As is customary in occupational exposure assessment analyses, we truncated the estimated spore count distribution at 0.5 spores, corresponding to half the detection limit.²⁰

Covariates

We coded risk-factor variables corresponding to sample type (bedrail or floor) and room risk of contamination (low, medium, and high). Sample surface area was encoded as a categorical variable (small or large). To adjust for surface area in our multivariable²¹ models, we also encoded sample surface area in as a continuous, log₁₀-transformed variable, log₁₀ (m²). Finally, we also coded 3 clustering variables: sample pair identifier, room identifier, and ward identifier.

Statistical analyses

For descriptive analyses, we measured the proportion of samples that were positive for the binary outcome variables and the geometric (log₁₀) mean spore count for the continuous outcome variables across the 3 risk-factor variables.

For multivariable analyses, we used separate logistic mixed effects models for the presence versus absence of *C. difficile* by 16S qPCR, toxin B qPCR, and enrichment culture. We used negative binomial mixed effects models for the estimated spore count by

Table 1. Estimated Environmental Sample *Clostridium difficile* Positivity and Quantity Based on qPCR (16S target and Toxin B target) and Enrichment Culture From Environmental Samples Collected at a Tertiary Hospital

Sample Area	No.	Positivity (N, %)			Estimated Spore Count (Geometric Mean)	
		16S qPCR	Toxin B qPCR	Enrichment culture	16S qPCR	Toxin B qPCR
Total	48	31 (64.6)	19 (39.6)	21 (43.8)	13.8	1.9
Surface area						
Small	24	14 (58.3)	7 (29.2)	9 (37.5)	7.0	1.2
Large	24	17 (70.8)	12 (50.0)	12 (50)	27.0	3.0
Type						
Bedrail	24	10 (41.7)	5 (20.8)	6 (25)	2.7	0.9
Floor	24	21 (87.5)	14 (58.3)	15 (62.5)	69.4	4.3
Surface area and type						
Small bedrail	12	5 (41.7)	2 (16.7)	3 (25.0)	2.1	0.7
Large bedrail	12	5 (41.7)	3 (25.0)	3 (25.0)	3.5	1.1
Small floor	12	9 (75.0)	5 (41.7)	6 (50.0)	23.3	2.3
Large floor	12	12 (100)	9 (75.0)	9 (75.0)	206.5	8.2
Room Risk of Contamination^a						
Low	20	10 (50.0)	5 (25.0)	7 (35.0)	4.3	1.3
Medium	8	4 (50.0)	3 (37.5)	1 (12.5)	5.0	1.7
High	20	17 (85.0)	11 (55.0)	13 (65.0)	65.6	3.0

NOTE. qPCR, quantitative polymerase chain reaction.

^aLow, occupant had no receipt of antibiotics in prior 14 days; medium, occupant had receipt of antibiotics in prior 14 days; high, occupant had confirmed *C. difficile* infection.

16S qPCR and toxin B qPCR.²² All multivariable models included the same fixed effects and random intercepts. We used fixed effects for patient risk stratum, sample type, and sample surface area, and we used random intercepts corresponding to the ward, the patient room, and the sample pair. The random intercepts for patient room and sample pair accounted for the clustering inherent to the split-plot sampling design.^{14,15} All multilevel models were fit using Bayesian random-effects regression models in the R statistical programming language (rstanarm library) and default settings (4 chains of 1,000 warmup and 1,000 sampling draws, and default weak priors²³). Furthermore, 95% confidence intervals (95% CIs) were based on the sampling draws.

We conducted 2 secondary analyses. First, to assess whether the association between sample surface area and yield was consistent across the surface type (bedrail vs floor), we ran an additional negative binomial multivariable model that added the interaction term between surface area and type. Second, to assess the relatedness of the 3 different laboratory techniques for measuring *C. difficile*, we measured the association between 16S qPCR spore counts and toxin B qPCR spore counts using linear regression, and between 16S qPCR spore count and enrichment culture positivity using logistic regression.

Ethics

This study was approved by the Public Health Ontario and Sunnybrook Health Sciences Center research ethics boards.

Results

Of the 48 samples collected, 31 (64.6%) were positive for *C. difficile* by 16S qPCR, with a geometric mean of 13.8 spores per sample; 19 (39.6%) were positive by toxin B qPCR, with a geometric mean of 1.9 spores per sample; and 21 (43.8%) were positive by enrichment culture.

Descriptive analyses

Large surface-area samples were more likely to be positive and had higher estimated spore counts compared to small surface-area samples (Table 1, Appendix 2). By 16S qPCR, 17 of 24 large surface-area samples (70.8%) were positive, while 14 of 24 small surface-area samples (58.3%) were positive. The geometric mean estimated spore count was 27.0 for large surface-area samples and 7.0 for small surface-area samples. However, large surface-area bedrail samples (0.23 m²) were less likely to be positive (5 of 12, 41.7%) than small surface-area floor samples (0.10 m²; 9 of 12, 75%) (Fig. 1). Overall, 21 of 24 floor samples (87.5%) were positive compared to 10 of 24 bedrail samples (41.7%), and the geometric mean estimated spore count was 69.4 for floor samples compared to 2.7 for bedrail samples.

Multivariable analyses

After multivariable adjustment, each 10-fold increase in surface area was associated with a 4.5-fold (adjusted odds ratio [aOR],

4.5; 95% CI, 0.79–34) increase in the odds of positivity and a 6.6-fold (adjusted count ratio [aCR], 6.6; 95% CI, 3.2–13) increase in the spore count (Table 2). Floor surfaces were 17-fold (OR, 17; 95% CI, 2.1–170) more likely to be positive than bed rails and had 27-fold (aCR, 27; 95% CI, 4.9–181) more spores. Compared to rooms of patients that received no antibiotics in the previous 14 days, samples from rooms of patients with *C. difficile* infection were 17-fold (aOR, 17; 95% CI, 1.2–246) more likely to be positive, with 11-fold (aCR, 11; 95% CI, 0.55–164) more spores. Models for toxin B qPCR and enrichment culture returned analogous findings. A sensitivity analysis that considered separate surface-area effects for bedrail and floor pairs by 16S qPCR spore count revealed that each 10-fold increase in surface area was

associated with a 3.7-fold (95% CI, 1.3–9.8) increase in bedrail spore count and a 9.0-fold (95% CI, 4.2–19.9) increase in floor spore count ($P = .08$).

Comparison of 16S qPCR versus toxin B qPCR and enrichment culture

Estimated spore counts by 16S qPCR were strongly associated with toxin B spore counts (Pearson’s ρ , 0.75) (Fig. 2, panel A) and with enrichment culture-based results. When the 16S spore count was 0, only 1 of 19 enrichment culture samples (5.3%) were positive. For comparison, 8 of 16 samples (50%) were culture positive for spore counts of 1 to 100; 7 of 8 samples (87.5%) were culture positive for spore counts of 100 to 1000; and 5 of 5 samples (100%) were culture positive for spore counts > 1,000 ($P_{\text{LogisticTrend}} < .001$) (Fig. 2, panel B).

Ribotyping

We successfully ribotyped 20 of 21 samples (95%) that were positive by enrichment culture, and we detected 8 different ribotypes (Table 3). Single ribotypes were identified in 16 of 20 samples (80%), and 2 ribotypes were identified in 4 of 20 samples (20%). Ribotype 015 was found in 11 of 12 positive samples (92%) from ward B, but 0 of 8 samples (0%) were positive from ward A ($P_{\chi^2} = .04$). Of 20 samples, 17 (85%) contained ribotypes known to have toxin A and B genes.

Discussion

In this study, we considered the impact of increasing sample surface area on *C. difficile* recovery using culture and culture-independent techniques. We found that increased sample surface area was associated with increased counts of spores recovered and increased proportions of samples that were positive by all laboratory techniques. Additionally, we identified that 16S qPCR, toxin B qPCR, and enrichment culture are all acceptable techni-

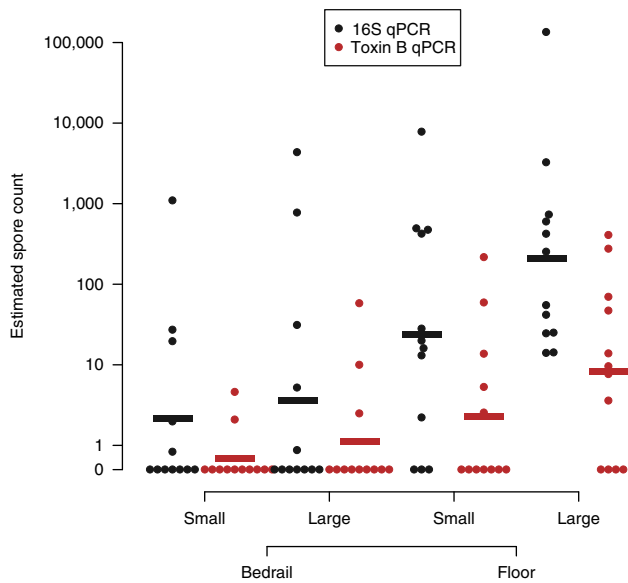


Fig. 1. Estimated *C. difficile* quantity based on quantitative PCR (16S target and Toxin B target). A horizontal bar is used to indicate the group geometric mean.

Table 2. Predictors of Environmental Sample *Clostridium difficile* Positivity and Quantity Based on qPCR (16S target and Toxin B target) and Enrichment Culture From Environmental Samples Collected at a Tertiary-Care Hospital, Based on Logistic and Linear Multilevel Regression Models

Sample Area	Positivity (Odds Ratio, 95% CI)			Estimated Spore Count (Count Ratio, 95%CI)	
	16S qPCR	Toxin B qPCR	Enrichment culture	16S qPCR	Toxin B qPCR
Surface area^a					
Per 10× increase	4.5 (0.79–34)	14 (1.4–284)	3.9 (0.67–28)	6.6 (3.2–13)	5.9 (1.6–24)
Type					
Bedrail	Reference	Reference	Reference	Reference	Reference
Floor	17 (2.1–170)	8.3 (0.6–143)	10 (1.3–116)	27 (4.9–181)	11 (1.2–130)
Room Risk of Contamination^b					
Low	Reference	Reference	Reference	Reference	Reference
Medium	1.1 (0.04–23)	1.5 (0.04–48)	0.28 (0.01–7.9)	0.95 (0.03–26)	1.3 (0.04–36)
High	17 (1.2–246)	6.9 (0.27–165)	13 (0.86–229)	11 (0.55–164)	2.9 (0.17–45)

NOTE. qPCR, quantitative polymerase chain reaction; CI, confidence interval.

^aFor multivariable analyses, sample surface area was coded as $\log_{10}(\text{m}^2)$ so that the effect estimate represents the odds ratio or count ratio per 10-fold increase in the surface area.

^bLow, occupant had no receipt of antibiotics in prior 14 days; medium, occupant had receipt of antibiotics in prior 14 days; high, occupant had confirmed *C. difficile* infection.

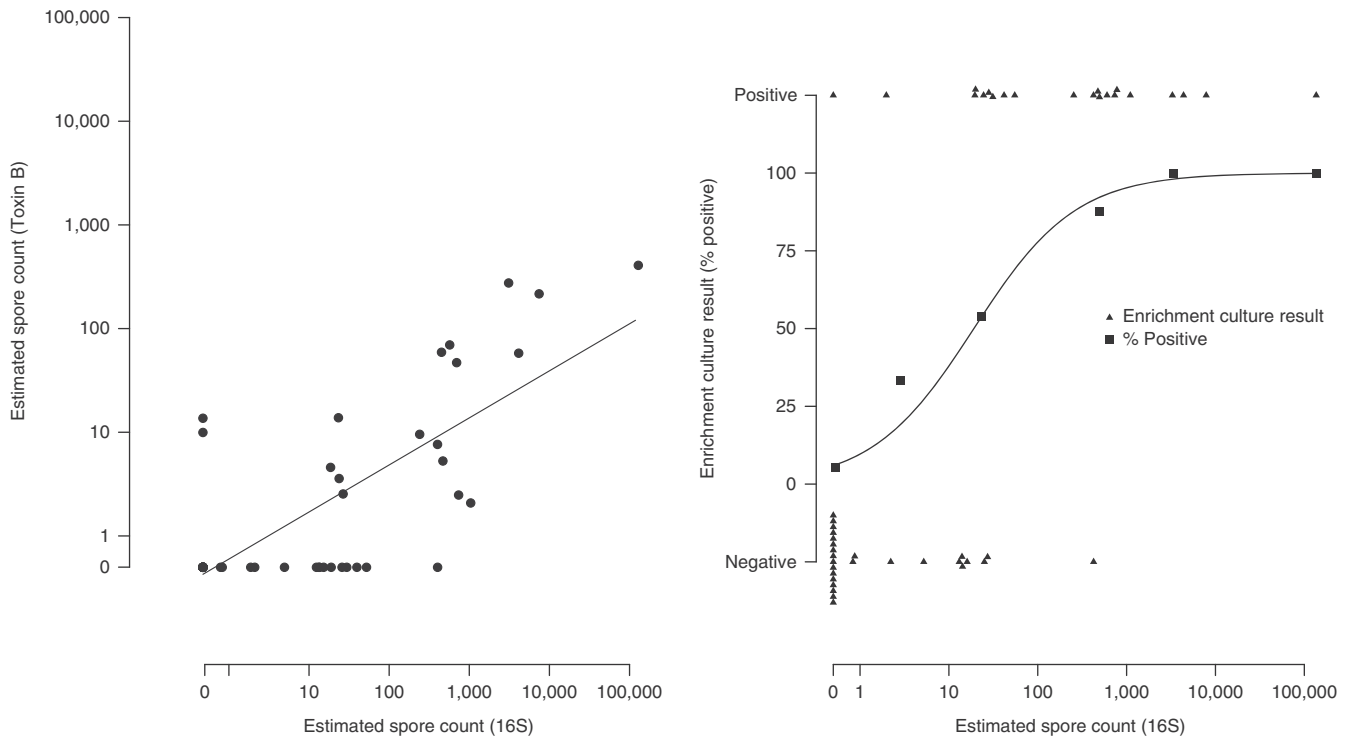


Fig. 2. Association between the testing methods. Panel A: 16S qPCR and Toxin B qPCR. Panel B: 16S qPCR and enrichment culture.

ques for measurement of environmental *C. difficile*. We also found that *C. difficile* contamination was common on bedrails and floors and was significantly more common in the rooms of patients with *C. difficile* infection.

Several techniques have been used to measure environmental *C. difficile* contamination, including culture,²⁴ enrichment culture,²⁵ and qPCR.¹⁸ Culture-based techniques are commonly used and inexpensive, but they can be plagued by low detection limits, issues of nonculturability, and long turnaround times for results. Although qPCR is a more sensitive and culture-independent technique, it is also more labor intensive and costly, largely due to the DNA extraction step. However, it can produce results within 2–3 hours. qPCR is routinely used for clinical diagnostics and has also been adapted for environmental sampling.¹⁸ We found that 16S qPCR was more sensitive than toxin B qPCR. We also found that results from qPCR-based techniques and culture-based techniques were strongly correlated. Increasing levels of contamination by 16S qPCR were strongly associated with increased levels by toxin B qPCR and enrichment culture-based positivity. At levels beyond 1,000 spores by 16S qPCR, all toxin B qPCR samples and enrichment culture samples were positive, suggesting that toxin B qPCR and enrichment culture may have been negative due to a lack of sensitivity of the laboratory technique rather than a lack of toxigenic or live cells on those surfaces.

Using real-world environmental samples, this study demonstrated that large surface-area samples yielded higher positivity and higher counts of spores across several different microbiologic techniques and 2 surface types. These findings provide further evidence of the advantages of maximizing surface area when conducting environmental sampling. For 16S qPCR, the effect of surface area on *C. difficile* capture may have been moderately stronger among floor samples than among bedrail samples. This finding may have been due to inconsistencies in the sampling of bedrails because bedrails have complex surfaces.

Previous studies have shown that, for *Bacillus atrophaeus*, large surface-area sampling (1 m²) translates into greater sensitivity for detection and necessitates fewer samples for testing exposure–outcome associations, compared with samples with smaller surface areas.²⁶ A more recent study used large surface area samples (up to 0.22 m²) to quantify the microbial bioburden of several hospital pathogens.⁹ Our work showed that all techniques tested, including both culture and culture-independent techniques, reacted positively to increased sample size, though the clinical relevance of environmental *C. difficile* burden has not yet been established.

We found that *C. difficile* contamination was ubiquitous in the hospital rooms we sampled. All rooms, even those of patients without a history of *C. difficile* or antibiotic use, yielded positive samples by 16S qPCR. It has been shown that floor-surface samples are approximately twice as likely to be culture positive as bedrail samples,⁶ and surfaces more proximate to patients are more likely to be contaminated than those farther from patients.²⁷ In our study, floor surfaces actually had 10 times more *C. difficile* contamination than bedrail surfaces. Also, 100% of the 12 large floor samples we collected were contaminated with *C. difficile* according to 16S qPCR. *Clostridium difficile* on floor surfaces may be a risk to patients because microbes on the floor may be transported within wards by shoe soles²⁸ are continually resuspended by foot traffic.²⁹ Furthermore, mobile patients can contaminate linens and themselves via their feet or shoes.¹³ Although our results do not allow us to assess directionality, the finding of strong relatedness of strains recovered within wards suggests that spatial dissemination involving floors may be a concern. These results also lend support to several studies that have shown the importance of ward-level effects for the transmission of *C. difficile* and other hospital-associated infections.^{30–32}

The infective dose of *C. difficile* for a healthy but susceptible inpatient is not known³³ and as such, safe levels of environmental *C. difficile* are not known. For mice, the environmental infectious

Table 3. Characteristics of *Clostridium difficile* Samples Successfully Ribotyped (N = 20)

Ward-Room ^a	Room Risk of Contamination ^b	Type	Surface Area	Ribotypes	Estimated Spore Count	
					16S qPCR	Toxin B qPCR
A-2	High	Floor	Small	027, PR18099	28.1	2.5
A-2	High	Floor	Large	027	424.2	7.7
A-2	High	Bedrail	Small	027, PR18101	19.6	4.6
A-2	High	Bedrail	Large	027	778.0	2.5
A-4	High	Floor	Large	PR18099	41.8	0.2
A-4	High	Bedrail	Small	014-0	2.0	0
A-5	Low	Floor	Small	400	0	0
A-5	Low	Floor	Large	400	55.1	0
B-7	Low	Floor	Large	015	24.6	13.9
B-8	Low	Floor	Small	015	475.6	59.4
B-8	Low	Floor	Large	015	602.3	69.9
B-11	High	Floor	Small	015	7,863.5	217.4
B-11	High	Floor	Large	015	136,143.6	409.5
B-11	High	Bedrail	Small	015	1,101.1	2.1
B-11	High	Bedrail	Large	015	4,373.7	58.1
B-10	Low	Floor	Small	015	19.9	0
B-10	Low	Floor	Large	015, 009	254.0	9.6
B-12	High	Floor	Small	015	495.1	5.3
B-12	High	Floor	Large	010	3,276.0	276.7
B-12	High	Bedrail	Large	015, 010	31.2	0

NOTE. qPCR, quantitative polymerase chain reaction.

^aNo positive samples were obtained from ward C.

^bLow, occupant had no receipt of antibiotics in prior 14 days; medium, occupant had receipt of antibiotics in prior 14 days; high, occupant had confirmed *C. difficile* infection.

dose required to infect 50% of mice (ID₅₀) for *C. difficile* was 1 hour of exposure to 5–10 culturable spores per square centimeter of cage floor.³⁴ Future research should seek to identify the infectious dose for *C. difficile* for both healthy individuals and those at a risk of *C. difficile* infection due to antibiotic receipt and to define the maximum threshold of environmental density of *C. difficile* that confers an acceptably low risk of infection. Such environmental density thresholds could be used to better ascertain the acceptability of current hospital cleaning protocols.

Our study has several limitations. First, it is a study of rooms in a single hospital over 2 days of sampling and may not be generalizable to other hospitals. We lacked several covariates including antibiotic history prior to hospital admission and timing of most recent cleaning prior to sample collection. However, these limitations were controlled for by the experimental design of our study, wherein sample surface area and sample type, our 2 exposures of primary interest, were compared to each other within rooms at the same point in time. Second, our small surface-area bedrail samples were selected from the head of the bedrail, while our large surface-area samples were chosen from the remainder of the bedrail, which could have led to a bias if spore density was higher or lower near the head of the bedrail.

In this study, we found that increased sample surface area was associated with increased counts of spores recovered and increased proportions of samples that were positive. We also found that *C. difficile* contamination was common on hospital bedrails and floors and even more so in the rooms of patients with *C. difficile* infection. Future studies attempting to quantify the environmental density of *C. difficile* should consider using large surface-area samples whenever possible. Further study is required to better understand the role of contaminated hospital floors in the transmission of *C. difficile* infection.

Supplementary materials. To view supplementary material for this article, please visit <https://doi.org/10.1017/ice.2018.103>

Acknowledgments. The authors thank Kwaku Adomako and Arezou Saedi for assistance with research ethics approval, sample collection, and transportation, and Victoria Williams for assistance with coordinating sample collection.

Financial support. This study was funded by a grant from the Canadian Institutes for Health Research. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Conflicts of interest. All authors declare no conflicts of interest relevant to this article.

References

1. Slimings C, Riley TV. Antibiotics and hospital-acquired *Clostridium difficile* infection: update of systematic review and meta-analysis. *J Antimicrob Chemother* 2014;69:881–891.
2. Smits WK, Lyras D, Lacy DB, Wilcox MH, Kuijper EJ. *Clostridium difficile* infection. *Nat Rev Dis Primer* 2016;2:16020.
3. Vardakas KZ, Trigkidis KK, Boukouvala E, Falagas ME. *Clostridium difficile* infection following systemic antibiotic administration in randomised controlled trials: a systematic review and meta-analysis. *Int J Antimicrob Agents* 2016;48:1–10.
4. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis* 2006;6:130.
5. Sethi AK, Al-Nassir WN, Nerandzic MM, Bobulsky GS, Donskey CJ. Persistence of skin contamination and environmental shedding of *Clostridium difficile* during and after treatment of *C. difficile* infection. *Infect Control Hosp Epidemiol* 2010;31:21–27.
6. Samore MH, Venkataraman L, DeGirolami PC, Arbeit RD, Karchmer AW. Clinical and molecular epidemiology of sporadic and clustered cases of nosocomial *Clostridium difficile* diarrhea. *Am J Med* 1996;100:32–40.
7. Weber DJ, Rutala WA, Miller MB, Huslage K, Sickbert-Bennett E. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, *Clostridium difficile*, and *Acinetobacter* species. *Am J Infect Control* 2010;38:S25–S33.
8. Hospodsky D, Yamamoto N, Peccia J. Accuracy, precision, and method detection limits of quantitative PCR for airborne bacteria and fungi. *Appl Environ Microbiol* 2010;76:7004–7012.
9. Shams AM, Rose LJ, Edwards JR, et al. Assessment of the overall and multidrug-resistant organism bioburden on environmental surfaces in healthcare facilities. *Infect Control Hosp Epidemiol* 2016;37:1426–1432.
10. Ali S, Muzslay M, Wilson P. A novel quantitative sampling technique for detection and monitoring of *Clostridium difficile* contamination in the clinical environment. *J Clin Microbiol* 2015;53:2570–2574.
11. Engelhardt NEP, Foster NF, Hong S, Riley TV, McGeachie DB. Comparison of two environmental sampling tools for the detection of *Clostridium difficile* spores on hard bathroom surfaces in the hospital setting. *J Hosp Infect* 2017;96:295–296.
12. Huslage K, Rutala WA, Sickbert-Bennett E, Weber DJ. A quantitative approach to defining “high-touch” surfaces in hospitals. *Infect Control Hosp Epidemiol* 2010;31:850–853.
13. Rashid T, Vonville H, Hasan I, Garey KW. Mechanisms for floor surfaces or environmental ground contamination to cause human infection: a systematic review. *Epidemiol Infect* 2017;145:347–357.
14. Altman N, Krzywinski M. Points of significance: split plot design. *Nat Methods* 2015;12:165–166.
15. Jones B, Nachtsheim CJ. Split-plot designs: what, why, and how. *J Qual Technol* 2009;41:340–361.
16. Ontario Agency for Health Protection and Promotion, Provincial Infectious Diseases Advisory Committee. *Best Practices for Environmental Cleaning for Prevention and Control of Infections in All Health Care Settings, 2nd revision*. Toronto, Canada; 2012.
17. *Surface Sampling Procedures for Bacillus Anthracis Spores from Smooth, Non-Porous Surfaces*. Washington, USA: The National Institute for Occupational Safety and Health; 2012.
18. Mutters R, Nonnenmacher C, Susin C, Albrecht U, Kropatsch R, Schumacher S. Quantitative detection of *Clostridium difficile* in hospital environmental samples by real-time polymerase chain reaction. *J Hosp Infect* 2009;71:43–48.
19. Kilic A, Alam MJ, Tisdell NL, et al. Multiplex real-time PCR method for simultaneous identification and toxigenic type characterization of *Clostridium difficile* from stool samples. *Ann Lab Med* 2015;35:306.
20. Ogden TL. Handling results below the level of detection. *Ann Occup Hyg* 2010;54:255–256.
21. Hidalgo B, Goodman M. Multivariate or multivariable regression? *Am J Public Health* 2013;103:39–40.
22. Guthrie KA, Gammill HS, Kamper-Jørgensen M, et al. Statistical methods for unusual count data: examples from studies of microchimerism. *Am J Epidemiol* 2016;184:779–786.
23. Stan Development Team. rstanarm: Bayesian applied regression modeling via Stan. R package version 2.13.1. Stan website. <http://mc-stan.org/>. Published 2016. Accessed April 23, 2018.
24. Buggy BP, Wilson KH, Fekety R. Comparison of methods for recovery of *Clostridium difficile* from an environmental surface. *J Clin Microbiol* 1983;18:348–352.
25. Nerandzic MM, Donskey CJ. Effective and reduced-cost modified selective medium for isolation of *Clostridium difficile*. *J Clin Microbiol* 2009;47:397–400.
26. Buttner MP, Cruz P, Stetzenbach LD, Klima-Comba AK, Stevens VL, Emanuel PA. Evaluation of the biological sampling kit (BiSKit) for large-area surface sampling. *Appl Environ Microbiol* 2004;70:7040–7045.
27. Moore G, Muzslay M, Wilson APR. The type, level, and distribution of microorganisms within the ward environment: a zonal analysis of an intensive care unit and a gastrointestinal surgical ward. *Infect Control Hosp Epidemiol* 2013;34:500–506.
28. Rashid T, Vonville HM, Hasan I, Garey KW. Shoe soles as a potential vector for pathogen transmission: a systematic review. *J Appl Microbiol* 2016;121:1223–1231.
29. Qian J, Peccia J, Ferro AR. Walking-induced particle resuspension in indoor environments. *Atmos Environ* 2014;89:464–481.
30. Brown K, Valenta K, Fisman D, Simor A, Daneman N. Hospital ward antibiotic prescribing and the risks of *Clostridium difficile* infection. *JAMA Intern Med* 2015;175:626–633.
31. Dubberke ER, Reske KA, Olsen MA, et al. Evaluation of *Clostridium difficile*-associated disease pressure as a risk factor for *C difficile*-associated disease. *Arch Intern Med* 2007;167:1092–1097.
32. Tosas Auguet O, Stabler RA, Betley J, et al. Frequent undetected ward-based methicillin-resistant *Staphylococcus aureus* transmission linked to patient sharing between hospitals. *Clin Infect Dis* 2018;66:840–848.
33. Weese JS. *Clostridium difficile* in food—innocent bystander or serious threat? *Clin Microbiol Infect* 2010;16:3–10.
34. Lawley TD, Clare S, Deakin LJ, et al. Use of purified *Clostridium difficile* spores to facilitate evaluation of health care disinfection regimens. *Appl Environ Microbiol* 2010;76:6895–6900.