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A Novel Quantitative Sampling Technique for Detection and Monitoring of *Clostridium difficile* Contamination in the Clinical Environment

Shanom Ali, Monika Muzslay, Peter Wilson

University College Hospital, Clinical Microbiology and Virology, London, United Kingdom

The horizontal transmission of *Clostridium difficile* in the hospital environment is difficult to establish. Current methods to detect *C. difficile* spores on surfaces are not quantitative, lack sensitivity, and are protracted. We propose a novel rapid method to detect and quantify *C. difficile* contamination on surfaces. Sponge swabbing was compared to contact plate sampling to assess the *in vitro* recovery of *C. difficile* ribotype 027 contamination (~10^0, 10^1, or 10^2 CFU of spores) from test surfaces (a bed rail, a stainless steel sheet, or a polypropylene work surface). Sponge swab contents were concentrated by vacuum filtration, and the filter membrane was plated onto selective agar. The efficacy of each technique for the recovery of *C. difficile* from sites in the clinical environment that are touched at a high frequency was evaluated. Contact plates recovered 19 to 32% of the total contamination on test surfaces, whereas sponge swabs recovered 76 to 94% of the total contamination, and contact plates failed to detect *C. difficile* contamination below a detection limit of 10 CFU/25 cm^2 (0.4 CFU/cm^2). In use, contact plates failed to detect *C. difficile* contamination (0/96 contact plates; 4 case wards), while sponge swabs recovered *C. difficile* from 29% (87/301) of the surfaces tested in the clinical environment. Approximately 74% (36/49) of the area in the vicinity of the patient was contaminated (~1.34 ± 6.88 CFU/cm^2 *C. difficile* spores). Reservoirs of *C. difficile* extended to beyond the areas near the patient: a dirty utility room sink (2.26 ± 5.90 CFU/cm^2), toilet floor (1.87 ± 2.40 CFU/cm^2), and chair arm (1.33 ± 4.69 CFU/cm^2). *C. difficile* was present on floors in ~90% of case wards. This study highlights that sampling with a contact plate may fail to detect *C. difficile* contamination and result in false-negative reporting. Our sponge sampling technique permitted the rapid and quantitative measurement of *C. difficile* contamination on surfaces with a sensitivity (limit, 0 CFU) greater than that which is otherwise possible. This technique could be implemented for routine surface hygiene monitoring for targeted cleaning interventions and as a tool to investigate routes of patient-patient transmission in the clinical environment.

Clostridium difficile infection (CDI) remains one of the most clinically important health care-associated infections and continues to burden health care services and finances (1, 2). The presentation of the disease can vary from mild diarrhea to severe colitis (3) and toxic dilatation that requires colectomy or may result in death. The use of proton pump inhibitors, residence in long-term-care facilities, and use of antibiotics are recognized risk factors (4, 5). However, the increasing incidence of community-acquired CDI suggests that other contributory factors may be responsible for the spread of the disease (6, 7). The horizontal transmission of CDI is implicated in patients with or without diarrhea, who may shed spores of *C. difficile* to contaminate their surrounding surfaces (8). Spores of *C. difficile* can persist on hospital surfaces for months (9), posing a risk of spreading to surfaces beyond the near patient environment, such as via the hands of patients, visitors, and health care workers (10).

To reduce the risk of transmission, patients considered to be sources of CDI should be isolated upon presentation of symptoms of diarrhea, and an enhanced cleaning protocol should be implemented (11). Decontamination of surfaces in the clinical environment using a sporicide is recommended for *C. difficile*-positive cases only (11). However, patients may remain asymptomatic, even though they are excreting *C. difficile* (3), and will not prompt the implementation of infection control measures in the absence of diarrhea. Consequently, patients with asymptomatic cases of *C. difficile* disease may continue to contaminate the clinical environment unhindered unless a carrier status is diagnosed.

Assessment of surfaces in the patient environment to monitor contamination levels and the persistence of *C. difficile* spores is not routinely performed. Current methods to sample surfaces for *C. difficile* contamination are not quantitative, have a low sensitivity, and require lengthy incubation periods. Routes for transmission between patients are not clear (12), and without effective surface monitoring, the horizontal transmission of *C. difficile* in the clinical setting is difficult to establish.

In this study, a novel method to detect *C. difficile* contamination on surfaces in the patient environment is proposed. The sampling technique was assessed both *in vitro* and in the clinical setting and demonstrated the quantitative measurement of *C. difficile* contamination on surfaces with a sensitivity (limit, 0 CFU) greater than that which is otherwise available. The rapid turnaround time of this method should accelerate identification of contaminated areas in the clinical environment and prompt the implementation

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Address correspondence to Shanom Ali, shanom.ali@uclh.nhs.uk.

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TABLE 1 Amount of *C. difficile* detected on various surfaces in the clinical environment using a contact plate or a sponge swab[a]

Zone and sample point description	Brazier's contact plate[b]		Sponge swab		
	Mean no. of CFU/cm^2 ± SD	% positive sites (no. of positive sites/total no. sampled)	Area sampled/cm^2 (approximate dimensions or description)[c]	Mean no. of CFU/cm^2 ± SD	% positive sites (no. positive sites/total no. sampled)
Patient room/area					
Floor corner	0	0 (0/5)	225 (15 by 15 cm)	0.87 ± 1.45	85.7 (12/14)
Bed rail/footboard	0	0 (0/5)	180 (3 by 60 cm)	0.17 ± 0.35	77.8 (7/8)
Bed control panel	0	0 (0/5)	225 (15 by 15 cm)	0.33 ± 0.74	70.0 (7/10)
Nurse call button	0	0 (0/5)	50 (25 cm^2 [front and back])	0.18 ± 0.19	81.8 (9/11)
Bedside table	0	0 (0/5)	300 (15 by 20 cm)	0.01 ± 0.03	66.7 (6/9)
Chair arm	0	0 (0/5)	150 (5 by 30 cm)	1.33 ± 4.69	63.6 (7/11)
Bin lid	0	0 (0/5)	120 (10 by 12 cm)	0.38 ± 1.29	40.0 (4/10)
Door handle (inner handle only)	0	0 (0/5)	50 (whole handle)	0.05 ± 0.13	60.0 (3/5)
Door handle (outer handle only)	0	0 (0/5)	50 (whole handle)	0	0.0 (0/5)
Patient bathroom					
Toilet floor (around bowl)	0	0 (0/5)	225 (15 by 15 cm)	1.87 ± 2.40	90.0 (9/10)
Toilet assist bar (upward-facing point)	0	0 (0/5)	150 (3 by 50 cm)	0.17 ± 0.32	42.9 (3/7)
Toilet flush	0	0 (0/5)	50 (entire flush handle)	0.01 ± 0.02	16.7 (1/6)
Toilet seat/commode seat	0	0 (0/5)	800 (10 by 80 cm)	0.76 ± 1.76	57.1 (4/7)
Tap handle/lever	0	0 (0/5)	50 (hot and cold levers)	0	0.0 (0/6)
Door handles (inner and outer combined)	0	0 (0/5)	100 (both handles)	0	0.0 (0/8)
Nurse/doctor station					
Table/desk surface	0	0 (0/5)	625 (25 by 25 cm)	0.01 ± 0.02	6.7 (1/15)
Computer keyboard	0	0 (0/5)	675 (45 by 15 cm)	0.01 ± 0.01	20.0 (3/15)
Dirty utility (sluice) room					
Sluice waste disposal/bin lid	0	0 (0/4)	120 (10 by 12 cm)	0.18 ± 0.39	16.7 (2/12)
Sluice room sink	0	0 (0/4)	225 (15 by 15 cm)	2.26 ± 5.90	25.0 (3/12)
Door handles (inner and outer combined)	0	0 (0/4)	100 (both handles)	0.18 ± 0.53	16.7 (2/12)
Laundry area, laundry basket (handlebars)	0	0 (0/4)	50 (2.5 by 20 cm)	0.08 ± 0.13	20.0 (2/10)
Total		0/96			87/301

[a] All contaminating numbers are displayed as the number of CFU per unit area (square centimeters).
[b] Contact plates were used for spot sampling of 25-cm^2 areas only.
[c] Sponge swabs were used to sample various surface areas.

of a targeted cleaning intervention(s). Our sampling technique could be implemented for routine surface hygiene monitoring and as a tool to investigate routes of patient-patient transmission in the clinical environment.

MATERIALS AND METHODS

Sampling of clinical environment. (i) Selection of sampling points in clinical environment. Sampling was performed at a teaching hospital in London, United Kingdom. A total of 21 sampling points that represent sites with a high frequency of contact in patient isolation rooms and the en suite bathrooms as well as sites in the nurse/doctor station and dirty utility (sluice) rooms of the test ward were selected (Table 1).

(ii) Sampling technique. For each case, all 21 sampling points were sampled by using either a 25-cm^2 contact plate (Brazier's cycloserine-cefoxitin-egg yolk [CCEY] agar; SGL Ltd., United Kingdom) or by wiping the entire test area (in a left-to-right motion, followed by wiping at 45° and 90° angles; the process was repeated 3 times) using a 25-cm^2 sponge swab premoistened with neutralizing solution (Lab M Ltd., United Kingdom). During the course of the study, each test (case) room was selected for sampling (using either technique) only once and thereafter was excluded.

(iii) Processing of sponge swabs. Sponge swabs were placed aseptically into sterile stomacher bags (VWR International, United Kingdom) containing 50 ml neutralizing solution (0.1% [wt/vol] sodium thiosulfate, 3% [wt/vol] Tween 80, 0.3% [wt/vol] lecithin [Sigma-Aldrich, United Kingdom]) prepared in phosphate-buffered saline (PBS) solution prest-erilized (by autoclaving at 121°C for 15 min) before use. The contents of each stomacher bag were homogenized manually by vigorously massaging the bag between the fingertips for 1 min and incubated at ambient temperature for 10 min. The total volume of homogenized solution in the stomacher bag was passed through a filter membrane (47 mm; nitrocellulose; pore size, 0.45 μm; Advantec, Toyo Roshi Kaisha, Japan) and plated aseptically onto Brazier's CCEY agar (Oxoid, Basingstoke, United Kingdom).

All Brazier's agar plates and Brazier's contact plates were incubated at 37°C under anaerobic conditions for 48 h prior to reading of the results. Presumptive *C. difficile* isolates were determined by colony morphology (examination of plates for flat, circular colonies yellow/yellow-brown in color with filamentous edges) and microscopy (Gram-positive rods) and confirmed to be *C. difficile* using *C. difficile* selective latex agglutination assays (Oxoid, Basingstoke, United Kingdom).

***In vitro* validation of sampling technique. (i) Preparation of test surfaces.** A polypropylene-laminated medium-density fiberboard work surface, a stainless steel sheet (grade 304), and a hospital bed rail (polypropylene M2; Evolution 150; Hill Rom, Ashby de la Zouch, United King-

TABLE 2 *C. difficile* spores recovered from various contaminated surfaces when sampled with a contact plate or using a novel sponge swabbing technique

Sampling technique	Inoculum (no. of CFU)	*C. difficile* spores recovered[a]					
		Polypropylene work surface		Stainless steel sheet		Bed rail	
		No. of CFU of spores	% spores recovered	No. of CFU of spores	% spores recovered	No. of CFU of spores	% spores recovered
Contact plate ($n = 6$)	$\sim10^2$	215 (208–237)	23.9 (20.2–26.3)	235 (107–250)	26.2 (16.3–27.8)	178 (107–232)	19.8 (12.5–25.7)
	$\sim10^1$	23 (1–45)	25.6 (4.7–49.7)	28 (4–42)	31.6 (16.7–46.7)	21 (8–36)	23.9 (11.4–40.6)
	$\sim10^0$	0	0	0 (0–1)	0.0 (0.0–16.7)	0	0
Sponge swab ($n = 10$)	$\sim10^2$	779 (740–812)	86.5 (82.3–90.2)	768 (669–800)	85.5 (74.3–88.9)	720 (586–835)	80.0 (65.1–92.8)
	$\sim10^1$	84 (76–89)	93.3 (84.7–98.9)	80 (72–91)	88.3 (80.0–99.5)	68 (64–86)	76.1 (70.6–95.3)
	$\sim10^0$	9 (8–11)	94.4 (88.9–116.7)	9 (7–11)	94.4 (77.8–111.4)	8 (6–11)	83.3 (69.9–116.7)

[a] *C. difficile* spores recovered represent the number per 25-cm^2 test area when the area was inoculated with approximately 10^0 CFU, 10^1 CFU, or 10^2 CFU of spores. Data represent medians (IQRs).

dom) were marked with 25-cm^2 test areas (i.e., 5-cm by 5-cm squares). Before each experiment the test surface was scrubbed with a nonabrasive sponge pretreated with a chlorine-free detergent, rinsed three times with boiling (~100°C) distilled water (DW), and then disinfected with a 70% ethanol solution and left to air dry. This in-house-validated cleaning protocol has been shown to consistently reduce microbial numbers to below detectable levels (data not shown).

(ii) Preparation of stock *C. difficile* spores. Suspensions of *C. difficile* spores were prepared and stored on the basis of methods previously described (13). *C. difficile* (a ribotype 027 clinical isolate) cultures from Brazier's agar plates (in duplicate) were harvested using a 10-μl loop and transferred to 1 ml of sterile distilled water. Aliquots (0.1 ml) from the suspension were surface plated onto eight blood agar plates (Columbia blood agar [CBA]; Oxoid, Basingstoke, United Kingdom) and incubated anaerobically at 37°C for 5 days (±4 h). *C. difficile* cultures from the CBA plates were harvested using a sterile cotton-tipped swab, transferred to a 10-ml solution of 70% ethanol prepared in sterile PBS (Oxoid, Basingstoke, United Kingdom), and incubated at ambient temperature for 48 h. The resulting spore suspensions were washed three times by centrifugation at 3,000 rpm (~1,500 × *g*) for 10 min and resuspended in 10 ml sterile DW. The presence of spores (phase-bright cells) was confirmed by phase-contrast microscopy, and the suspension was determined to be free (~95%) of organic debris. The titers of the stock *C. difficile* spore suspensions were confirmed by plate culture and adjusted (by combining several spore preparations) to produce an inoculum dose of approximately 10^6 CFU/ml. Final spore suspensions were stored in 10-ml solutions of 50% ethanol prepared in sterile PBS.

(iii) Preparation of test suspensions of *C. difficile* spores. Before each experiment, *C. difficile* spore suspensions were centrifuged at 3,000 rpm for 10 min (~1,500 × *g*) and resuspended in 10 ml sterile distilled water (control soil) or in synthetic feces (5% [wt/vol] tryptone, 5% [wt/vol] bovine serum albumin, 0.4% [wt/vol] mucin [type II from porcine stomach]; Sigma-Aldrich, United Kingdom) prepared in sterile PBS. All soil suspensions were filter sterilized by passage through a nitrocellulose membrane filter (pore size, 0.45 μm; VWR International, United Kingdom) and stored in a refrigerator (2 to 5°C) prior to use.

(iv) *In vitro* assessment of sampling technique. One milliliter of the stock suspension was transferred to 9 ml sterile synthetic soil solution, and the procedure was repeated to produce serial dilutions of 1/100, 1/1,000, and 1/10,000 of the initial stock concentration. Ten-microliter aliquots of each dilution were dispensed onto test areas on each surface type to dose ~10^2, 10^1, and 10^0 CFU and were spread using an L-shaped spreader. Inoculated surfaces were incubated under ambient conditions for 1 h prior to sampling of each test area using either a contact plate or a sponge swab (folded in half twice; area, approximately 6.25 cm^2) and processed as described above.

Statistics. Means ± standard deviations were compared using a standard *t* test, and nonparametric data were compared using a Mann-Whitney U test. One-tailed tests were used for all analyses, and differences were considered statistically significant when P was <0.05.

RESULTS

***C. difficile* in clinical environment. (i) Contact plate sampling.** Four rooms that had previously been occupied with CDI-positive (symptomatic) patients were sampled using Brazier's *C. difficile*-selective contact plates. *C. difficile* contamination was not detected on any of the sites sampled with a contact plate (0/96 sites).

(ii) Sponge swab sampling. A total of 301 sites in 18 rooms (15 occupied, 3 unoccupied) were sampled using the sponge swab technique. Only nine of the patients (9/15; 60%) in these rooms were positive for *C. difficile* infection at the time of sampling, and six of these patients were symptomatic with diarrhea.

Up to 74% (36/49) of the surfaces in the area near the patient (bed rail, bed control panel, nurse call button, bedside table, patient chair arm) were contaminated with *C. difficile* (mean, 1.34 ± 6.88 CFU/cm^2; Table 1). The dirty utility (sluice) room sink (2.26 ± 5.90 CFU/cm^2), patient toilet floor (1.87 ± 2.40 CFU/cm^2), and patient chair arm (1.33 ± 4.69 CFU/cm^2) were the most highly contaminated surfaces in the clinical ward. *C. difficile* contamination was present on approximately 90% of the floors in the patient en suite bathrooms (1.87 ± 2.40 CFU/cm^2) and in the corner of the floor of the patient isolation rooms (0.87 ± 1.45 CFU/cm^2). Reservoirs of *C. difficile* contamination were also identified in nonpatient areas: on the computer keyboard at the nurse/doctor station (0.01 ± 0.01 CFU/cm^2), the door handles of the dirty utility (sluice) room (0.18 ± 0.53 CFU/cm^2), and the handlebars of the laundry basket for clean linen (0.08 ± 0.13 CFU/cm^2).

In-house validation of the sampling technique. The largest bioburden dose (10 μl) seeded onto test surfaces was approximately 900 ± 90 CFU *C. difficile* spores (~10^2), and tests were repeated with 10-fold (~10^1) and 100-fold (10^0) dilutions on each test material (a polypropylene work surface, a stainless steel sheet, and a hospital bed rail surface). The numbers of *C. difficile* spores recovered from these surfaces using either a contact plate or sponge swab are shown in Table 2.

When surfaces were sampled with a contact plate ($n = 6$), *C. difficile* spores could be recovered from surfaces only when the contamination was greater than 10 CFU/25 cm^2. The percentage

of *C. difficile* spores recovered from each surface ranged from 19.8 to 31.6% when using a contact plate. When the bioburden was between 10 and 100 CFU/25 cm^2, there was no difference between the numbers or percentages of *C. difficile* spores recovered from either surface type (bed rail, a stainless steel sheet, or a polypropylene work surface) using a contact plate ($P > 0.05$).

In contrast, the sponge swab technique ($n = 10$) could recover *C. difficile* from contaminated surfaces even when the bioburden was less than 10 CFU spores. The recovery of *C. difficile* from contaminated surfaces was higher when the sponge swab technique was used than when the contact plate was used ($P < 0.05$). Regardless of the surface type, the sponge swab technique consistently recovered between 76 and 94% of the contaminating spores.

DISCUSSION

The use of an effective sampling technique is essential for accurate reporting of the microbial bioburden on surfaces in the clinical environment. The use of contact plates is a popular choice for sampling, as sampling can be performed rapidly (i.e., it requires only that the contact plate be pressed onto the target area) prior to incubation. However, this method can be used only for spot sampling, as it is limited to the surface area of the contact plate used. Sponge swabs have been used for the sampling of surfaces in the patient environment (14, 15). The ability to apply pressure during manual swabbing with a sponge allows the operator to recover *C. difficile* contamination, both vegetative cells and spores, more effectively, and the sponge swab may be manipulated around the contours of the test surface to contact a range of points greater than the number that would be reached with the contact plate, which would otherwise be missed when the contact plate (which has an area of approximately 25-cm^2) is used.

Investigations to determine the reservoirs of *C. difficile* in the hospital environment have been attempted previously (12, 16–18). However, these methods were limited to determining the presence or absence of *C. difficile* spores on various surfaces. The ability to determine numbers of *C. difficile* spores on a surface allows the differentiation of areas posing a high or low risk of cross contamination. However, the sampling protocol used may limit the ability to accurately measure the level of contamination on a surface. Sampling with a contact plate can be used to assign a quantitative (numerical) value to indicate the level of *C. difficile* bioburden on the surfaces assessed, though the numbers recovered may not be representative of the contamination level present. Recovery of *C. difficile* contamination may be improved by sampling with a sponge swab. However, several studies have subjected sponge swabs to enrichment in nutrient-rich medium before they are plated on selective agar (14, 15). Consequently, enrichment limits an assessment to a qualitative result to denote only the presence/absence of *C. difficile* on surfaces.

In this study, we developed a sponge swab technique to improve the isolation of *C. difficile* from the clinical environment and from sites with a high frequency of contact in rooms occupied by patients with or without CDI. The elution of *C. difficile* cells or spores captured in sponge swabs and passage of the resulting solution through a filter membrane before plating on selective agar conferred three main benefits. First, the primary advantage of our sampling protocol is the ability to generate quantitative (numerical) readings of the *C. difficile* bioburden, as opposed to the qualitative (presence/absence) results obtained by enrichment methods (14, 15, 19). Second, this technique is highly sensitive with a theoretical detection limit of 0 CFU. Third, the removal of the enrichment stage used in other studies (14, 15) reduced the turnaround time (which is typically 5 to 7 days) and enabled the quantitative measurement of *C. difficile* contamination present on surfaces within 2 days from the time of initial sampling. Further, the use of a 0.45-μm-pore-size membrane filter ensured that the pore size was adequate for the capture of most *C. difficile* bacteria and spores (which are 0.5 to 0.7 μm in size). Attempts to capture *C. difficile* on sponge swabs with a reduced enrichment stage (48 h) have been described (19), though this still requires a further culture stage (48 h) and cannot quantify contaminating numbers like the method proposed in this study can.

Supplementation of selective medium with lysozyme has been demonstrated to produce significant increases in the recovery of *C. difficile* from swabs taken from the patient environment (20). While the effect of supplementation on the detection of *C. difficile* was not evaluated in the current study, future assays could incorporate lysozyme to enhance recovery.

The clinical ward assessment highlights the limitation of the contact plate technique. Although *C. difficile* contamination was not isolated from any of the 96 sites (0/96 sites; 0%) sampled with a contact plate (Table 1), *C. difficile* contamination was recovered from 87/301 sites (28.9%) sampled using the sponge swab technique.

Our sponge swabbing technique identified that *C. difficile* contamination was not restricted to the patient environment: the nurse/doctor station desk and computer keyboards were contaminated with *C. difficile* in 6.7% and 20.0% of cases. All areas sampled in the dirty utility room tested positive for *C. difficile* in up to 25% of cases, while 20% of the clean linen trolleys were contaminated with *C. difficile* when sampled using our sponge sampling technique. An assessment of the same areas using a contact plate failed to identify the risks posed by the presence of *C. difficile* contamination on these surfaces.

The ability to recover *C. difficile* spores from the surfaces using contact plates, flocked swabs, or rayon swabs may be less when the surface contamination is low (21).

In the current study, sampling with the contact plate failed to detect *C. difficile* on surfaces during the in-house validation assays when the contamination level was below 10 CFU/25 cm^2, suggesting a lower limit of detection equivalent to 0.4 CFU/cm^2. Nonetheless, our sponge swab technique was able to recover *C. difficile* from 76% (16/21) of the surfaces in the clinical environment, despite a *C. difficile* bioburden of below 0.4 CFU/cm^2 (Table 1).

The sampling of *C. difficile* from surfaces using a contact plate is limited by manipulation of the plate over the test surfaces and the pressure that can be applied to recover a representative level of the contamination. Further, the presence of antibiotic supplements in the selective media used in contact plates may apply stresses upon *C. difficile* spores, resulting in further reductions in recovery (16). This is reflected in the low level of recovery (between 19.8 and 31.6%) of *C. difficile* from seeded surfaces demonstrated by our in-house validation (Table 2). In contrast, the sponge swab technique recovered up to 94.4% of *C. difficile* spores from seeded surfaces, regardless of the surface type.

Recent work demonstrated that *C. difficile* (ribotype 027) spores may persist on surfaces for up to 60 min, despite disinfection with a commercially available sporicide (22). The presence of residual disinfectant on surfaces over this period may negate the efficacy of a real-time sampling protocol. In the current study, the

neutralizing solution used was validated in-house (23) to demonstrate efficacy at least 10 min postcleaning against a range of commercially available disinfectants (sodium dichloroisocyanurate-, chlorine dioxide-, peroxyacetic acid-, and hypochlorous acid-based products). Our protocol therefore may be used for the routine monitoring/auditing of cleaning practices and used to direct further decontamination in areas demonstrating poor compliance.

A screening program has been implemented at our hospital to monitor *C. difficile* contamination levels in the clinical environment. Our sampling protocol has been shown to detect *C. difficile* from the area near a patient 3 days before a patient develops symptoms of CDI (24). Ribotyping of both the patient and environmental isolates found that the strains were indistinguishable (ribotype 002). Adoption of protocols similar to the sponge sampling technique described here for the quantitative surveillance of *C. difficile* on surfaces would be invaluable and offer faster reporting of contaminated high-risk areas to facilitate the control of *C. difficile* transmission in the clinical environment.

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REFERENCES

1. **Public Health England.** 2014. *C. difficile* infections: quarterly counts by acute trust and CCG and financial year counts and rates by acute trust and CCG. *In Clostridium difficile*: guidance, data and analysis. Public Health England, London, United Kingdom. https://www.gov.uk/government/statistics/clostridium-difficile-infection-annual-data.
2. **McDonald LC, Owings M, Jernigan DB.** 2006. *Clostridium difficile* infection in patients discharged from US short-stay hospitals 1996–2003. Emerg Infect Dis **12:**409–415. http://dx.doi.org/10.3201/eid1203.051064.
3. **Johnson S, Gerding DN.** 1998. *Clostridium difficile*-associated diarrhea. Clin Infect Dis **26:**1027–1036. http://dx.doi.org/10.1086/520276.
4. **McFarland LV.** 2009. Evidence-based review of probiotics for antibiotic-associated diarrhea and *Clostridium difficile* infections. Anaerobe **15:**274–280. http://dx.doi.org/10.1016/j.anaerobe.2009.09.002.
5. **Dubberke ER, Wertheimer AI.** 2009. Review of current literature on the economic burden of *Clostridium difficile* infection. Infect Control Hosp Epidemiol **30:**57–66. http://dx.doi.org/10.1086/592981.
6. **Pituch H.** 2009. *Clostridium difficile* is no longer just a nosocomial infection or an infection of adults. Int J Antimicrob Agents **33:**42–45. http://dx.doi.org/10.1016/S0924-8579(09)70016-0.
7. **O'Connor JR, Johnson S, Gerding DN.** 2009. *Clostridium difficile* infection caused by the epidemic BI/NAP1/027 strain. Gastroenterology **136:**1913–1924. http://dx.doi.org/10.1053/j.gastro.2009.02.073.
8. **Donskey CJ.** 2013. *Clostridium difficile*—beyond the usual suspects. N Engl J Med. **369:**1263–1264. http://dx.doi.org/10.1056/NEJMe1310454.
9. **Kramer A, Schwebke I, Kampf G.** 2006. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis **6:**130. http://dx.doi.org/10.1186/1471-2334-6-130.
10. **Gerding DN, Johnson S, Peterson LR, Mulligan ME, Silva J.** 1995. Clostridium difficile-associated diarrhea and colitis. Infect Control Hosp Epidemiol **16:**459–477.
11. **Department of Health.** 2008. *Clostridium difficile* infection: how to deal with the problem. *In Clostridium difficile*: guidance, data and analysis. Public Health England, Department of Health, London, United Kingdom. https://www.gov.uk/government/publications/clostridium-difficile-infection-how-to-deal-with-the-problem.
12. **Eyre DW, Cule ML, Wilson DJ, Griffiths D, Vaughan A, O'Connor L, Ip CL, Golubchik T, Batty TEM, Finney JM, Wyllie DH, Didelot X, Piazza P, Bowden R, Dingle KE, Harding RM, Crook DW, Wilcox MH, Peto TEA, Walker AS.** 2013. Diverse sources of *Clostridium difficile* infection identified on whole-genome sequencing. N Engl J Med **369:**1195–1205. http://dx.doi.org/10.1056/NEJMoa1216064.
13. **Shetty N, Srinivasan S, Holton J, Ridgway GL.** 1999. Evaluation of microbicidal activity of a new disinfectant: Sterilox 2500 against *Clostridium difficile* spores, *Helicobacter pylori*, vancomycin resistant *Enterococcus* species, *Candida albicans* and several *Mycobacterium* species. J Hosp Infect **41:**101–105. http://dx.doi.org/10.1016/S0195-6701(99)90046-4.
14. **Otter JA, Havill NL, Adams NM, Cooper T, Tauman A, Boyce JM.** 2009. Environmental sampling for *Clostridium difficile*: swabs or sponges? Am J Infect Control **37:**517–518. http://dx.doi.org/10.1016/j.ajic.2009.01.005.
15. **Hill KA, Collins J, Wilson L, Perry JD, Gould FK.** 2013. Comparison of two selective media for the recovery of *Clostridium difficile* from environmental surfaces. J Hosp Infect **83:**164–166. http://dx.doi.org/10.1016/j.jhin.2012.10.006.
16. **Malik DJ, Patel KV, Clokie MRJ, Shama G.** 2013. On the difficulties of isolating *Clostridium difficile* from hospital environments. J Hosp Infect **84:**181–183. http://dx.doi.org/10.1016/j.jhin.2013.02.020.
17. **Warrack S, Duster M, Van Hoof S, Schmitz M, Safdar N.** 2014. *Clostridium difficile* in a children's hospital: assessment of environmental contamination. Am J Hosp Infect **42:**802–804. http://dx.doi.org/10.1016/j.ajic.2014.03.008.
18. **Verity P, Wilcox MH, Fawley W, Parnell P.** 2001. Prospective evaluation of environmental contamination by *Clostridium difficile* in isolation side rooms. J Hosp Infect **49:**204–209. http://dx.doi.org/10.1053/jhin.2001.1078.
19. **Best EL, Parnell P, Thirkell G, Verity P, Copland M, Else P, Denton M, Hobson RP, Wilcox MH.** 2014. Effectiveness of deep cleaning followed by hydrogen peroxide decontamination during high *Clostridium difficile* incidence. J Hosp Infect **87:**25–33. http://dx.doi.org/10.1016/j.jhin.2014.02.005.
20. **Wilcox MH, Fawley WN, Parnell P.** 2000. Value of lysozyme agar incorporation and alkaline thioglycollate exposure for the environmental recovery of Clostridium difficile. J Hosp Infect **44:**65–69. http://dx.doi.org/10.1053/jhin.1999.0253.
21. **Claro T, Daniels S, Humphreys H.** 2014. Detecting *Clostridium* spores from the inanimate surfaces of the hospital environment: which method is best? J Clin Microbiol **52:**3426–3428. http://dx.doi.org/10.1128/JCM.01011-14.
22. **Ali S, Moore G, Wilson APR.** 2011. Spread and persistence of *Clostridium difficile* spores during and after cleaning with sporicidal disinfectants. J Hosp Infect **79:**97–98. http://dx.doi.org/10.1016/j.jhin.2011.06.010.
23. **Sutton SVW, Proud DW, Rachui S, Brannan DK.** 2002. Validation of microbial recovery from disinfectants. PDA J Pharm Sci Technol **56:**255–266.
24. **Ali S, Manuel R, Wilson P.** 2014. Diverse sources of *C. difficile* infection. N Engl J Med **370:**182–184. http://dx.doi.org/10.1056/NEJMc1313601.