MAJOR ARTICLE

The Potential for Airborne Dispersal of *Clostridium difficile* from Symptomatic Patients

Emma L. Best,[1] Warren N. Fawley,[1] Peter Parnell,[1] and Mark H. Wilcox[1,2]

[1]Microbiology Department, Old Medical School, Leeds General Infirmary, Leeds Teaching Hospitals National Health Service Trust, and [2]University of Leeds, Leeds, United Kingdom

(See the editorial commentary by Donskey, on pages 1458–1461.)

Background. The high transmissibility and widespread environmental contamination by *Clostridium difficile* suggests the possibility of airborne dissemination of spores. We measured airborne and environmental *C. difficile* adjacent to patients with symptomatic *C. difficile* infection (CDI).

Methods. We conducted air sampling adjacent to 63 patients with CDI for 180 h in total and for 101 h in control settings. Environmental samples were obtained from surfaces adjacent to the patient and from communal areas of the ward. *C. difficile* isolates were characterized by ribotyping and multilocus variable-number tandem-repeat analysis to determine relatedness.

Results. Of the first 50 patients examined (each for 1 h), only 12% had positive air samples, most frequently those with active symptoms of CDI (10%, vs 2% for those with no symptoms). We intensively sampled the air around 10 patients with CDI symptoms, each for 10 h over 2 days, as well as a total of 346 surface sites. *C. difficile* was isolated from the air in the majority of these cases (7 of 10 patients tested) and from the surfaces around 9 of the patients; 60% of patients had both air and surface environments that were positive for *C. difficile.* Molecular characterization confirmed an epidemiological link between airborne dispersal, environmental contamination, and CDI cases.

Conclusions. Aerosolization of *C. difficile* occurs commonly but sporadically in patients with symptomatic CDI. This may explain the widespread dissemination of epidemic strains. Our results emphasize the importance of single-room isolation as soon as possible after the onset of diarrhea to limit the dissemination of *C. difficile.*

Clostridium difficile infection (CDI) is a major burden to health care facilities [1], with increasing rates since 2002 in the United States [2], Canada [3–5], and Europe [6, 7]. *C. difficile* is transmissible between hospitalized patients, and control measures to limit cross-infection are part of routine practice. Pragmatically, it is desirable to nurse patients with CDI in isolation, although there is a lack of robust evidence to support the utility of single rooms in preventing transmission. However, limited availability of single rooms in some settings can lead to the frequent management of CDI cases in open wards [8, 9]. With the recognition and emergence of virulent strains associated with CDI outbreaks, such as ribotype 027/NAP1 [1–7], it has become increasingly important to determine how transmission is occurring and to establish effective interventions to minimize these risks.

It has been estimated that a patient with CDI can excrete between 1×10^4 and 1×10^7 of *C. difficile* per gram of feces [10]. *C. difficile* spores may be resistant to disinfectants and can survive for months or years on contaminated surfaces [11–14]. Environmental contamination with *C. difficile* spores occurs at as many as 34%–58% of sites despite cleaning, with surfaces of fomites being most frequently contaminated [15–17]. Crucially, the hands of health care workers are significantly more likely to be positive for *C. difficile* if the environment is heavily contaminated with the bacterium [18].

There are few data to substantiate the risks associated with airborne transmission of *C. difficile* in hospitals. A number of studies have alluded to the possibility that *C. difficile* could potentially spread through the air [15,

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Reprints or correspondence: Prof Mark H. Wilcox, Microbiology Dept, Old Medical School, Leeds General Infirmary, Leeds LS1 3EX, West Yorkshire, United Kingdom (mark.wilcox@leedsth.nhs.uk).

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19–22]. We aimed to determine the extent of *C. difficile* contamination in ward environments by recovery of *C. difficile* from air and environmental surfaces in the immediate vicinity of patients with symptomatic CDI. We aimed to establish an epidemiological link between patients and their surroundings, using highly discriminatory DNA fingerprinting, as well as the relationship between near-patient activity and *C. difficile* aerosolization.

METHODS

Organization of Air Sampling

Approval for the study was obtained from the Leeds Teaching Hospitals' Research Committee. The first phase of the investigation (6 months) comprised air sampling (total, 50 h) for 1 h adjacent to 50 patients with confirmed CDI (including 13 in general medical wards, 25 in elderly care wards, and 12 in the *C. difficile* ward, of whom 43 were in single rooms and 7 were in multioccupied bays). All air was maintained by standard ventilation. The second and third phases of sampling (4 months) involved 10 h of air sampling over 2 days (total, 130 h for proven CDI cases [see below]) and 40 environmental samples per patient in elderly care wards and the *C. difficile* ward; 19 of 20 patients in these phases were in single rooms. The second phase of sampling comprised 10 patients with CDI identified by nursing staff as having suspected cases (not laboratory confirmed). The third phase of sampling focused on 10 patients with symptomatic (cytotoxin-positive) CDI.

Control air sampling consisted of the following. During phase 1, 11 air samples (11 h) were obtained adjacent to patients without a history or symptoms suggestive of CDI. During phase 2, 7 of the 10 patients with suspected CDI proved to be cytotoxin negative and were therefore counted as control tests (70 h). During phase 3, air sampling was conducted adjacent to a patient without a history of CDI (10 h) and in the corridor of the same ward (10 h). Air sampling totaled 180 and 101 h for CDI case patients and control subjects. During sampling, we observed and recorded ongoing activities that could be associated with marked air disturbance (eg, bed changing and frequency of visitors). Routine environmental cleaning was done each morning, using a detergent/chlorine (1000 ppm) agent.

Air Sampling

Air was collected using 1 of 2 AirTrace Environmental portable samplers (Biotrace; Microbial Contamination Control). Air sampling was conducted as close as practically possible to the patient's bed, via a 2-m Tygon tube (Saint-Gobain) placed within 1 m at the foot of the bed. As the air entered the sampler (28.3 L/min), it was forced through a fine slit (44×0.152 mm) at a velocity of 70 m/s, thereby causing particulate matter (minimum size, 0.4 μm) to impact on a *C. difficile* selective agar plate. Plates (140-mm diameter) contained Brazier's cycloserine–cefoxitin–egg yolk agar (Bioconnections) supplemented with 5 mg/mL lysozyme (CCEYL) (not prereduced) [23]. The plate rotated constantly; thus, after culture the location of the colonies represented the time of recovery from air, making it possible to link it to activities near to the sampler. Plates were transported to the laboratory and incubated anaerobically (37°C for 48–72 h). After each sampling session or day, the machine was cleaned externally and internally with a sporicidal disinfectant (Trigene; Medichem).

Environmental Surface Sampling

Initial in vitro tests for determining the efficiency of Polywipe sponges. Polywipe sponges (Medical Wire & Equipment) were initially examined as a pilot to this investigation to determine their efficiency for recovery of *C. difficile* from environmental surfaces. In vitro tests (in triplicate) were conducted using 200-μL aliquots of diluted spore suspensions ($2.4–2.4 \times 10^5$) spread onto an aluminium surface (30×15 cm) and left to dry for 1 h. Duplicate test-seeded areas were sampled using both standard environmental swabs and sponges. Each sponge or swab tip was placed into a Stomacher bag (Seward) with 50 mL of Ringer solution and processed for 30 s. Aliquots were cultured on CCEYL and anaerobically enriched in cooked-meat fastidious anaerobe broth (E&O Laboratories) at 37°C for 48 h.

Patient environment sampling. Following the pilot study, sponges were used for environmental sampling (Table 1). After sampling, 50 mL of Ringer solution was added to each sponge, followed by 30-s processing in a Stomacher bag. Liquid from the bag was passed through a 0.45-μm filter (Millipore), which was then placed in 10 mL of cooked-meat broth and subcultured on CCEYL, as described above.

Culture of Patient Fecal Samples

Patient fecal samples (pea-sized aliquots) were alcohol-shocked by immersion in 1 mL of 50% ethanol solution. After being vortexed for 10 s and left to stand at room temperature for $\geqslant$1 h, samples were cultured on CCEYL plates, as described above.

Polymerase Chain Reaction Ribotyping and Multilocus Variable-Number Tandem-Repeat Analysis

Polymerase chain reaction ribotyping was performed on all *C. difficile* isolates as described previously [24]. Multilocus variable-number tandem-repeat analysis (MLVA) was conducted as described elsewhere [25], using 7 loci (A6, B7, C6, E7, F3, G8, and H9). Fragments were analyzed using GeneMapper software (version 4.0; Applied Biosystems), and copy numbers were determined. The summed absolute difference between 2 MLVA-typed isolates is the calculated summed tandem-repeat difference (STRD) at all 7 loci [26]. MLVA types with a STRD $\leqslant$2

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Table 1. Detail of 20 Surfaces Tested during Environmental Sampling Using Polywipe Sponges (Surface Area, ~30 cm^2)

Surface tested
Surfaces in vicinity of patient
Inside door handle
Outside door handle
Bed rails
Bedside table
Hand-wash sink
Clinical waste bin
Overbed light
Medical equipment
Commode
Floor (adjacent to patent bed)
Walking frame
Communal area surfaces tested
Ward door
Desk surface of nurses' station
Patient toilet
Patient sink
Patient bath
Sluice room door
Notes trolley
Store room door
Staff room door

were indicative of a high degree of genetic relatedness among *C. difficile* isolates.

RESULTS

Polywipe sponge in vitro tests. Sponges were significantly more effective than swabs ($P = .006$) at recovering *C. difficile* from surfaces (Table 2). In tests comprising 2400 colony-forming units (CFUs) spread on a test surface, a recovery of 52% with sponges versus zero with swabs was achieved. To increase the detection limit further (2 CFUs), an enrichment step was added, on the basis of previous experience with recovering *C. difficile* from environmental sites [23]. Sponges also allowed sampling of larger surface areas, and so these were used in preference to swabs during phases 2 and 3.

First-phase sampling of 50 patients with confirmed CDI. Of the first 50 patients examined (1 h), only 6 (12%) had positive cultures from the air sampling. There was a trend toward there being more positive air samples from patients with active diarrheal symptoms, compared with those without diarrheal symptoms (10% vs 2%; $P = .1$). Of the 5 symptomatic patients with positive air samples, 2 were in beds on different 6-bedded bays (fully occupied), and 3 were in single rooms. During air sampling, cleaning and bed making were taking place close to 2 of the positive patients. No *C. difficile* was recovered from any of the control air samples ($n = 11$).

Second-phase air and environmental sampling of symptomatic patients with suspected but unconfirmed CDI. Results from phase 1 suggested that airborne dissemination of *C. difficile* spores may be occurring before laboratory confirmation of CDI was obtained. Of 10 patients identified by nursing staff as having suspected CDI, only 3 proved to have laboratory confirmed cases. Patient U4, who had a positive air sample, was in a single room and underwent air sampling on 15 December 2008 and 16 December 2008, with CDI confirmed on 16 December 2008. *C. difficile* colonies were recovered on 3 occasions (day 1, at 1115, 1130, and 1315), corresponding to ward cleaning at 1130 and to curtain closure around the bed at 1315. Ribotyping and MLVA confirmed that indistinguishable isolates were recovered from the patient and air at each time point (ribotype 106; MLVA profile 23-13-23-2-6-4-2). Patients U2 and U5 were also confirmed to be positive for CDI, but no positive air or environmental samples were obtained. The remainder of the patients ($n = 7$) were confirmed to be negative for cytotoxin and were thus considered as control (albeit diarrheal) patients. A positive air sample was collected from one of these control patients (U8), and positive environmental samples were collected from patient U6 (commode) and from patient U9 (bed, floor, table, sink, and the ward storeroom handle). *C. difficile* with the same ribotype and MLVA type (027; 31-22-17-12-5-8-2) was obtained from the bed, floor, table, and sink, but *C. difficile* with a different ribotype and MLVA type (106; 24-14-22-2-6-4-2) (STRD, >10) was recovered from the storeroom door.

Third-phase air and environmental sampling of symptomatic patients with confirmed CDI. Of the 10 patients tested (10 h), 7 had at least 1 positive air sample, 4 on multiple occasions. The most common times when *C. difficile* was recovered from the air corresponded with activity close to patients (Figure 1). Between 1000 and 1100, a drinks delivery occurred for patients, which corresponded to the recovery of *C. difficile* from 2 patients. The peak at 1145 corresponded with ward rounds between 1100 and 1200. The second peak comprising a total recovery of 8 colonies (from 4 patients) corresponded to lunch delivery (1200) and visiting time (1200–1400).

In total, 346 environmental surface samples were obtained during this phase, of which 10% yielded *C. difficile* (from 9 of 10 patients). For the single patient without a positive environmental sample, *C. difficile* was recovered from the air. The highest levels of recovery of *C. difficile* were from surfaces closest to the patients and the areas frequently handled, including the patients' bed, bedside table, sink, and bin (6 or more isolations). There were fewer (<2) positive environmental samples from infrequently touched surfaces.

Six patients had *C. difficile*–positive environmental and air samples (Table 3). For 3 patients, *C. difficile* recovered from the air and at least 1 environmental sample had identical MLVA

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Table 2. Mean *Clostridium difficile* Counts from In Vitro Surface Recovery Tests for Polywipes and Swabs

No. of CFUs spread onto test surface	Mean CFUs recovered (n = 6)					
	Environmental swabs			Polywipe sponges		
	No. of colonies on plate	No. recovered	Percent recovery	No. of colonies on plate	No. recovered	Percent recovery
2.4×10^5	1	2500	1	82	2×10^5	83
24,000	1	2500	10	7	17,500	72
2400	…	…	…	0.5	1250	52
240	…	…	…	…	…	…
24	…	…	…	…	…	…
2.4	…	…	…	…	…	…

NOTE. The test surface area was 30 × 15 cm. CFUs, colony-forming units.

types. For the other 3 positive patients, the *C. difficile* recovered from the air in each environment was considered to be highly related to at least 1 environmental sample obtained within the same environment (either a single- or double-locus variant, with a STRD ≤6). It was also possible to confirm an epidemiological link for the *C. difficile* isolates from air, diagnostic fecal samples, and environmental surfaces. For example, for patient C1 the *C. difficile* isolates from feces, air (1045 and 1050), and a table were all ribotype 027, with highly related MLVA types and 6 identical loci (31-22-15-12-5-10-2 and 31-22-15-12-5-8-2; a single-locus variant with a STRD of 2). *C. difficile* was not recovered from 20 h of control air samples.

DISCUSSION

Airborne transmission and environmental contamination of *C. difficile* was first suspected in hamster experiments [27]. Studies reported significant contamination of objects in the immediate environment and suggested that airborne cross-contamination appeared to be less important than that from contact surfaces

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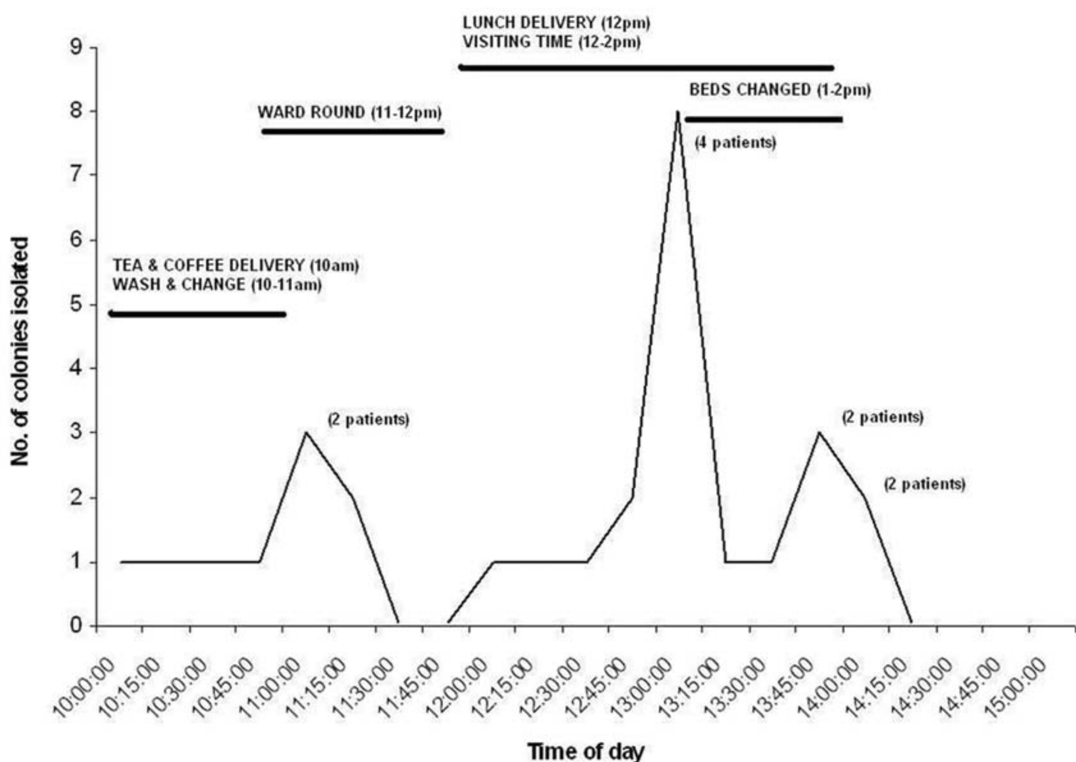

Figure 1. Line indicating the total number of *Clostridium difficile* colonies recovered at various times throughout the day (total of 10 patients tested for 2 days). The number of patients the colonies were isolated from is indicated in parentheses.

Table 3. Molecular Testing of Patient Samples and Phase 3 Positive Air and Environmental Samples for Symptomatic Patients with Confirmed *Clostridium difficile* Infection

Patient	Date tested	Date confirmed positive	Sample type (time of day)	Ribotype	MLVA result
C1	1/8/2008	1/2/2009	Air (1045)	027	31-22-15-12-5-10-2
			Air (1050)	027 (2 colonies)	31-22-15-12-5-10-2
			Air (1215)	027	31-22-15-12-5-8-2
			Air (1345)	027	31-22-15-12-5-8-2
			Air (1355)	027	31-22-16-12-5-8-2
			Table	027	31-22-15-12-5-10-2
			Feces	027	31-22-15-12-5-8-2
			Store handle	106	21-13-29-2-6-4-2
C2	2/5/2009	1/18/2009	Air (1245)	027 (2 colonies)	31-22-15-12-5-10-2
			Air (1300)	027, 001 (2 colonies)	27-28-31-9-5-9-2
			Air (1345)	027 (2 colonies)	31-19-33-9-5-12-2
			Feces	027	27-22-19-12-5-8-2
C3	2/17/2009	1/15/2009	Air (1010)	027	26-22-17-12-5-8-2
			Floor	027	31-22-18-12-5-8-2
			Sluice room door	106	24-14-22-2-6-4-2
	2/18/2009	1/15/2009	Bed	027	26-22-17-12-5-8-2
			Feces	027	31-22-18-12-5-8-2
C4	2/17/2009	2/18/2009	Air (1250)	106, 106 (2 colonies)	24-14-23-2-6-4-2
			Bed	106	24-14-22-2-6-4-2
			Table	106	24-14-22-2-6-4-2
			Commode	106	24-14-22-2-6-4-2
	2/18/2009	2/18/2009	Bed	110	24-14-22-2-6-4-2
			Commode	106	24-14-22-2-6-4-2
			Air (1140)	106	24-14-22-2-6-4-2
			Air (1240)	106	24-14-22-2-6-4-2
			Feces	106	24-14-22-2-6-4-2
C5	2/19/2009	2/16/2009	Air (1300)	106	24-13-24-2-6-4-2
			Air (1340)	106	24-13-24-2-6-4-2
			Door handle (outside)	106	24-14-24-2-6-3-2
			Bed	106	21-13-29-2-6-4-2
			Table	106	24-13-24-2-6-4-2
			Sink	106	24-13-24-2-6-4-2
			Bin	106	24-13-24-2-6-4-2
			Medical equipment	106	25-13-24-2-6-4-2
			Floor	106	24-13-24-26-4-2
	2/20/2009	2/16/2009	Air (1000)	106	24-13-24-2-6-4-2
			Air (1020)	106	24-13-24-2-6-4-2
			Air (1220)	106	24-13-24-2-6-4-2
			Air (1300)	106	24-13-24-2-6-4-2
			Table	106	24-13-24-2-6-4-2
			Bin	106	24-13-24-2-6-4-2
			Feces	106	24-13-24-2-6-4-2
C6	2/23/2009	2/14/2009	Bed	027	0-27-33-8-5-5-2
			Stool	027	26-14-31-6-7-0-4
C7	2/23/2009	2/24/2009	Sink	027	28-22-19-12-5-8-2
	2/24/2009	2/24/2009	Air (1050)	027	30-22-20-12-5-8-2
			Feces	027	30-22-20-12-5-8-2
C8	3/16/2009		Air (1115)	106 (2 colonies)	24-15-22-2-6-4-2
			Bin	106	24-15-22-2-6-4-2
	3/17/2009		Table	106	23-15-22-2-6-4-2
			Sink	174	24-15-22-2-6-4-2
			Bin	106	24-21-22-2-6-4-2

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Table 3. *(Continued.)*

Patient	Date tested	Date confirmed positive	Sample type (time of day)	Ribotype	MLVA result
			Walking frame	106	24-15-22-26-4-2
			Feces	106	24-15-22-2-6-4-2
C9	3/19/2009		Sink	106	25-13-23-2-6-5-2
			Feces	106	21-15-18-2-6-4-2
C10	3/23/2009		Bed	027	23-21-18-12-5-8-2
			Feces	027	29-22-18-12-5-8-2

NOTE. MLVA, multilocus variable-number tandem-repeat analysis.

[21, 27]. Fekety et al [22] used a slit-impaction air sampler and failed to isolate *C. difficile* from the air around patients with CDI but demonstrated extensive environmental and hand contamination. Roberts et al [19] recovered *C. difficile* from air using a cyclone air sampler in a hospital but did not recover *C. difficile* from associated environmental surfaces. Our study appears to be the first to recover *C. difficile* from the air and environment within the same time period and provide confirmation of a link between isolates.

We have shown that *C. difficile* is commonly (from 7 of 10 patients intensively studied) but sporadically present in the air close to symptomatic patients with CDI. By MLVA, we confirmed the presence of indistinguishable or very highly related strains of *C. difficile* in the environment, patient fecal specimens, and air. Several studies have confirmed the utility of MLVA for discrimination within *C. difficile* ribotypes [25, 28], including ribotype 106 (authors' unpublished data). Thus, our findings help explain the widespread dissemination of *C. difficile* in the hospital environment, including to infrequently touched or cleaned sites [11–13, 15, 16]. For example, we previously showed that 69% of infrequently touched ("high dust") surfaces were positive for *C. difficile* in an elderly medical ward within 6 months of ward opening [29]. We emphasize that without prolonged sampling of air we would have underestimated the sporadic nature of airborne *C. difficile* dispersal. This may explain the failure of some investigators to detect *C. difficile* in air [21, 22, 27].

We detected airborne *C. difficile* most commonly during periods of activity, particularly around the busy lunchtime period. These observations suggest that the air within the patient's immediate environment is contaminated with *C. difficile* spores either directly from symptomatic patients or from environmental surfaces and that people movement, including the opening and closing of doors, contributes to the circulation and dispersal of airborne *C. difficile*. It has been demonstrated previously that areas associated with much air movement, such as air vents, are contaminated with *C. difficile* [29, 30]. These findings have implications for cleaning practices. Unless cleaning is done frequently around symptomatic patients with CDI, including infrequently touched places, reaccumulation of *C. difficile* will occur on surfaces via the air. Disturbance of already-contaminated articles, such as the bin or bed linen, may contribute to spore aerosolization. It has been demonstrated previously that bed linen can be contaminated [31], and during this study we demonstrated that 3 of 30 bed curtains were culture positive for *C. difficile* on a ward with 2 confirmed CDI cases (authors' unpublished data). Therefore, activities known to liberate particles into the air, such as bed making and curtain drawing [32, 33], as well as contact with these items may contribute to the spread and aerosolization of *C. difficile*.

The environmental surface sampling results were consistent with previous studies showing that frequently touched areas are most often *C. difficile* positive [17, 31, 34]. *C. difficile* was not recovered from the least-touched areas, such as the light. The patient room door handles were very infrequently found to harbor *C. difficile*, which presumably reflects the frequent use of hand hygiene practices immediately before entering or leaving rooms. Conversely, *C. difficile* was recovered from sluice room door handles, likely reflecting contamination by staff disposing of feculent material. It might be expected to recover *C. difficile* from the patients' communal bathrooms [17, 31, 34]. However, such facilities were unlikely to be frequented by patients with CDI during this study, because they tended to use en-suite rooms and/or their own commodes.

There are some limitations to the present study. *C. difficile* recovery was generally modest, which may reflect methodological problems or a genuine low environmental microbial burden. The air sampler used was reliant on a slit-to-agar impaction method. A similar study [19] used a machine that recovered airborne material directly into solution but recorded airborne *C. difficile* counts that were orders of magnitude greater than our results. It is possible that positioning of the air sampler next to a toilet may have partly explained the higher airborne counts of *C. difficile* [19]. Because we sampled air close to patients for 5-h periods, we had to use the machine contained within a soundproof box and collect samples via an extension tube. The tube may have resulted in a loss of particulate matter collected onto the plates. The practicalities of

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prolonged sampling close to patients may have caused inconsistencies; for instance, during testing the air sampler or tube may have been moved because of visitors or patient care activities. A further difficulty was the timing and extent of symptoms, particularly because our phase 1 data suggested that air contamination by *C. difficile* was more likely while diarrhea was occurring. Although we had confirmation that patients were *C. difficile* toxin positive, we had to rely on health care staff to inform us of symptoms, and so sampling likely occurred at different times relative to the onset of CDI. For future studies, it would be useful to conduct air sampling before (this would also increase sampling of control subjects, which was limited in our study) and during the course of CDI to determine the frequency of *C. difficile* aerosolization in symptomatic patients.

It remains unclear whether the frequent presence of particular strains in health care environments reflects the burden of CDI caused by epidemic types or whether these have enhanced capacity to persist—for example, because of greater sporulation [30, 35, 36]. Nevertheless, our results suggest that there is a clear risk for *C. difficile* contamination via the air, particularly in patients with active CDI symptoms. The efficacy of these approaches as a control mechanism for CDI remains unproven. By contrast, the results of the present study do justify the use of single rooms for patients with suspected or proven CDI, even when such resources are limited [8]. In particular, we believe our findings underscore the importance of early patient isolation, as soon as possible after the onset of diarrhea and before laboratory diagnosis of CDI is confirmed. Allowing even a few hours before patient isolation or the wait until laboratory diagnosis is obtained, even with rapid tests, may not be adequate to prevent environmental dissemination of *C. difficile* via the air. Such a mechanism would at least partly explain the rapid spread and large outbreaks of CDI typified by epidemic strains, such as *C. difficile* ribotype 027 [1–7]. Recognition of the risk of airborne dissemination provides an opportunity to reduce transmission, especially of epidemic *C. difficile* strains.

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