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Potential for aerosolization of *Clostridium difficile* after flushing toilets: the role of toilet lids in reducing environmental contamination risk

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SUMMARY

Background: Toilet facilities in healthcare settings vary widely, but patient toilets are commonly shared and do not have lids. When a toilet is flushed without the lid closed, aerosol production may lead to surface contamination within the toilet environment.
Aim: To substantiate the risks of airborne dissemination of *C. difficile* following flushing a toilet, in particular when lids are not fitted.
Methods: We performed *in-situ* testing, using faecal suspensions of *C. difficile* to simulate the bacterial burden found during disease, to measure *C. difficile* aerosolization. We also measured the extent of splashing occurring during flushing of two different toilet types commonly used in hospitals.
Findings: *C. difficile* was recoverable from air sampled at heights up to 25 cm above the toilet seat. The highest numbers of *C. difficile* were recovered from air sampled immediately following flushing, and then declined 8-fold after 60 min and a further 3-fold after 90 min. Surface contamination with *C. difficile* occurred within 90 min after flushing, demonstrating that relatively large droplets are released which then contaminate the immediate environment. The mean numbers of droplets emitted upon flushing by the lidless toilets in clinical areas were 15–47, depending on design. *C. difficile* aerosolization and surrounding environmental contamination occur when a lidless toilet is flushed.
Conclusion: Lidless conventional toilets increase the risk of *C. difficile* environmental contamination, and we suggest that their use is discouraged, particularly in settings where CDI is common.

Introduction

Control measures to limit *Clostridium difficile* transmission in healthcare environments include barrier methods, isolation of infected patients and compliance with hand hygiene measures to minimize the risk of the dissemination of *C. difficile* spores.[1] Additionally, adherence to environmental cleaning and disinfection policies, including surfaces and equipment, have been shown to be important in reducing spore contamination and *C. difficile* infection (CDI) rates.[2,3] Recent work has demonstrated the potential for airborne dissemination of *C. difficile* spores, especially from patients with recent onset diarrhoea, and has suggested that this may contribute to widespread environmental contamination.[4,5] Despite implementation of these control measures, hospitals continue to

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experience CDI case clusters, prompting a search for additional ways to reduce environmental contamination.

Toilet facilities in healthcare settings vary widely, but patient toilets are commonly shared and do not have lids. When a toilet is flushed without the lid closed, aerosol production may lead to surface contamination within the toilet environment.[6–10] There are no data to substantiate the risks of airborne dissemination and environmental contamination by *C. difficile* following toilet flushing, in particular when lids are not fitted.

Methods

Toilet used for seeding experiments

The toilet room is in the Microbiology Department of Leeds Teaching Hospitals NHS Trust (LTHT), measures 8 m^2, contains two toilet cubicles (only one of which was used in the study), and was closed for normal use throughout the study. The toilet was a standard wash-down design (Armitage Shanks, Rugeley, UK) with a seat lid. Lidless or lidded versions of the same model are installed throughout patient, staff and public areas in LTHT. Before experiments, all inside and outside surfaces of the toilet were thoroughly cleaned/disinfected with Chlor-clean (1000 ppm of free available chlorine; Guest Medical, Edenbridge, UK), and then neutralized with sodium thiosulphate (VWR, Lutterworth, UK) to remove any residual chlorine. The toilet was then flushed four times to remove any traces of cleaning products. This procedure was also carried out to decontaminate the toilet between experiments. The floor and walls of the toilet cubicle used were also cleaned with Chlor-clean.

Preparation of faecal suspension

Human faeces were collected from five healthy elderly volunteers (>65 years) with no history of antimicrobial use in the two months before donation. The samples were confirmed as *C. difficile* culture-negative and then pooled to produce 100 g of faeces. Pooled faeces were emulsified in 1 L pre-reduced phosphate-buffered saline, stomached and coarse-filtered through sterile muslin to give a smooth faecal suspension and divided into 200 mL aliquots.

Preparation of C. difficile spores and inoculation of faecal suspension

Ten fresh blood agar plates were inoculated with *C. difficile* strain P24 (UK epidemic strain, polymerase chain reaction ribotype 001) and incubated anaerobically for 10 days. All growth was removed and resuspended in 1 mL sterile saline. An equal volume of absolute ethanol was added and the suspension was left for 1 h at room temperature to kill vegetative bacteria. Suspensions were centrifuged at 3000 *g* for 15 min and the pellet resuspended in 1 mL sterile water. Spore suspensions were enumerated on *C. difficile* selective agar and stored at 4 °C until use. Immediately before testing an aliquot of spore suspension was added to a 200 mL aliquot of faecal suspension to give a final spore concentration of 10^7 cfu/mL. Bacterial *C. difficile* counts were measured by serial dilution and culture on selective agar.

Air sampling

Air was collected using an AirTrace Environmental portable sampler (microbial contamination control) (Biotrace International plc, Bridgend, UK) via a 2 m Tygon tube (Saint-Gobain, Courbevoie, France). The end of the tube was clamped at one of three heights above the toilet bowl: toilet seat height, 10 cm above the seat and handle height (25 cm). As air enters the air sampler (28.3 L/min) it is 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. The plate rotated constantly, and thus after culture the location of the colonies represents the time of recovery from the air (e.g. relative to toilet flushing). Plates were transported to the laboratory and incubated anaerobically (37 °C for 48 h). After each test the machine was cleaned externally and internally with a sporicidal disinfectant (Trigene, Medichem International, Sevenoaks, UK) and run on a purge cycle.

Environmental testing

For each test, six *C. difficile* selective agar settle plates were placed around the toilet: top of the cistern, on the right- and left-hand side of the toilet seat (located on top of the lid for lid-closed tests) and three on the floor (15 cm in front of the toilet, on the left- and right-hand sides of the toilet). Plates were sited before flushing and remained in place throughout the 90 min testing time. Following testing, plates were transported to the laboratory and incubated as above.

Organization of testing

Experiments were performed to determine the extent of contamination of the air and environment following toilet flushing. Replicate experiments were performed to determine the magnitude of aerosol dissemination following flushing, with the lid open and closed, and with the air sampling tube at different heights. For each test the inoculated faecal suspension was poured into the toilet bowl and applied to the porcelain sides above the water line in order to mimic the effect of diarrhoea in the bowl. The air sampler was switched on and the toilet was flushed. To confirm the baseline microbiological status of the toilet and environment, six settle plates were placed in the room 24 h before testing began. Control tests were carried out before each series of tests and involved using sterile water instead of inoculated faecal suspension.

In-situ measurement of droplet emission

Separate experiments were carried out to determine the extent of droplet emission associated with toilet flushing. These included the toilet used in seeding experiments, and a further 10 different toilets in clinical areas of LTHT. One hundred millilitres of natural food colouring was added to the toilet bowl, and a sheet of cling film was stretched over the top of the toilet seat prior to toilet flushing. After flushing, the sheet of cling film was removed, placed on to a sheet of filter paper and transferred droplets were counted.

Results

Experiments to determine the extent of C. difficile contamination from lidless and lidded toilet following flushing.

Initial tests were carried out to determine the extent of *C. difficile* aerosolization following toilet flushing. In order to maximize the recovery of aerosolized *C. difficile*, the end of the air sampling tube was clamped in the centre of the toilet seat at the level of the toilet seat. The mean air count ($N = 4$ experiments) of *C. difficile* immediately following a flush was 36 cfu, the majority of which were recovered during the first 5 min; the air counts declined to 8 cfu after 60 min and 3 cfu after 90 min. *C. difficile* was not recovered from air in the control experiments where water was added to the bowl and the toilet flushed. With the toilet seat lid open, tests demonstrated that *C. difficile* could be recovered following a single flush with the end of the air sampling tube at seat level (12-fold greater counts than when lidded), but also at heights of 10 cm and 25 cm (Table I).

Environmental contamination of surfaces surrounding the toilet

With the lid closed, no *C. difficile* was recovered on the settle plates on any surface. By contrast, with the lid open, *C. difficile* was recovered on the settle plates at all locations ($N = 6$ experiments; mean 1–3 cfu per plate) except for the floor on the left-hand side. Similar counts were obtained in replicate experiments; in each case no *C. difficile* was recovered from the floor on the left-hand side, presumably reflecting the hydrodynamics of the toilet flush.

In-situ measurement of droplet emission

Upon flushing, droplets of varying size were ejected to the height of the seat in all toilets tested (Table II) (Figure 1). The mean number of droplets (11) emitted by the (Microbiology Department) toilet used for in-situ testing experiments was similar (15) to that observed for other toilets of the same style in the hospital (Figure 1a). Droplet counts for rimless toilets in the hospital were higher (mean 47) (Figure 1b).

Table I
Comparison of recovery of *Clostridium difficile* from the air with the toilet seat open and closed ($N = 2$)

Sample time	Mean cfu *C. difficile* detected in air samples 0–90 min after each flush					
	Control tests (water only added)	Toilet lid closed		Toilet lid open		
		10 cm above	Seat height	25 cm above	10 cm above	Seat height
0–30 min	0	4	3	7	6	35
30–60 min	0	1	7	4	0	3
60–90 min	0	0	0	1	0	0

Table II
Number of droplets ejected from toilets following a single flush

Location	Toilet style	Usage	No. of droplets[a]
Microbiology department	A	Staff	8
Microbiology department	A	Staff	13
Hospital ward	A	Staff	12
Hospital ward	A	Patient	7
Hospital ward	A	Patient	14
Hospital ward	A	Patient	16
Hospital ward	A	Patient	26
Hospital ward	B	Patient	55
Hospital ward	B	Patient	43
Hospital ward	B	Patient	61
Hospital ward	B	Patient	46
Hospital ward	B	Patient	29

Toilet style A, standard wash-down design; toilet style B, rimless pan with a raised seat.
[a] Represents the number of droplets of toilet bowl water collected on to cling film placed over the bowl at the toilet seat height following a single flush.

Discussion

Our study is the first to investigate the effect of a lid closure on the aerosolization and deposition of *C. difficile* associated with toilet flushing. Earlier studies investigated the aerosolization of microbes with considerably less survival potential than *C. difficile*. Using a domestic toilet seeded with *Serratia marcescens*, Darlow and Bale reported that bacteria carrying droplets produced by flushing a toilet remained airborne for up to 12 min, before settling on surfaces throughout a bathroom.[11] Later studies used coliform bacteria in domestic and hospital toilets and demonstrated aerolization and deposition of bacteria carrying particles on adjacent surfaces.[6,7] Gerba *et al.* showed that large numbers of *E. coli* or MS-2 phage remained in the bowl after flushing with the lid open due to the adsorption of organisms to the porcelain surfaces of the bowl; even continual flushing could not remove the bacteria.[8] Barker and Bloomfield carried out toilet seeding experiments using *Salmonella enteritidis* and were able to isolate the organism from the air using an air sampler following flushing with the lid open.[10]

Newsom concluded from a series of in-situ tests that the potential for environmental contamination by faecal bacteria associated with flushing of hospital toilets was low, based on the concentration of bacteria in faeces required to generate aerosolization in relation to those present during disease. However, neither anaerobes nor spore-forming bacteria were examined, as the study predated the first reports of human CDI. Notably, there was a 100-fold variation in the magnitude of airborne bacteria released when toilets were flushed, depending on which bacterial species was examined. Crucially, the faecal concentration of *C. difficile* can vary markedly in CDI, and explosive diarrhoea is not uncommon. Louie *et al.* reported that mean *C. difficile* faecal counts in 30 CDI cases were 7.0 $\log_{10} \pm 2.4$ per g (95% CI: 6.0–7.9).[12]

We simulated an episode of *C. difficile* diarrhoea when there is likely to be heavy contamination of both the internal toilet bowl and water. We used an inoculum of *C. difficile* spores representative of the average bacterial load present in

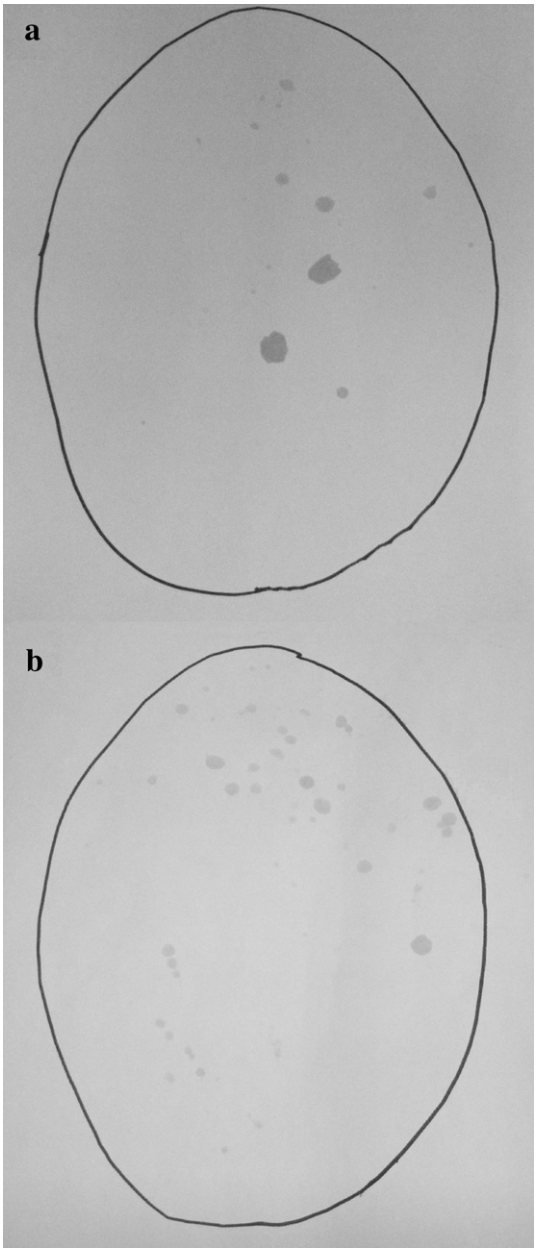

Figure 1. Filter paper impression to show droplets ejected during flushing on to cling film placed across a standard wash-down design toilet (a) and rimless pan with raised seat toilet (b).

CDI.[12] The highest levels of *C. difficile* were recovered immediately following flushing, and then declined 8-fold after 60 min and a further 3-fold after 90 min. The highest bacterial counts were detected from air sampled at the height of the toilet seat; however, it was still possible to recover *C. difficile* at heights of 10 cm and 25 cm above the toilet seat, demonstrating that water turbulence created during flushing can force droplets out of the toilet bowl and into the air. Closing the toilet seat lid markedly reduced the number of *C. difficile* recovered from air after flushing; there was a 10-fold reduction in recovered bacteria from air sampled at seat level. Low numbers of *C. difficile* were still recovered following flushing with the lid closed, suggesting that organisms were still aerosolized, most likely being forced out through gaps between the top of the toilet bowl and seat, and between the lid and the seat (15 and 10 mm, respectively).

C. difficile was recovered on settle plates placed on the floor, cistern and toilet seat during the 90 min after flushing, demonstrating that relatively large droplets are released and that these then contaminate the immediate environment. We confirmed the production of droplets upon flushing by visual inspection of cling film placed over toilets, which is consistent with other reports.[8–11] Closing the toilet seat lid was shown to prevent such large droplet aerosolization, as *C. difficile* was not recovered on any settle plates under such conditions. We have recently demonstrated that *C. difficile* aerosolization occurs commonly in patients with CDI, especially early in disease. We found that *C. difficile* spore-bearing particles fall ~1 m in 15 min.[13] This suggests that the majority of the *C. difficile* recovered on the settle plates in the present study was due to relatively quick deposition of *C. difficile* spores following flushing. Thus, surfaces can become rapidly seeded with *C. difficile* after toilet flushing (without a closed lid). Importantly, the resilient nature of spores means that only very frequent cleaning could be expected to remove such environmental contamination, especially in the context of repeated toilet use.

There are some limitations to the present study. We investigated only one toilet in detail and thus only one design. We believe that the toilet was correctly functioning, and note that there is no routine servicing of toilets, remedial work instead being dependent on fault reporting. Toilet bowl contents were rinsed away as expected, droplet measurements were consistent with the same design of toilets in clinical areas, and thus ostensibly the toilet used for seeding experiments was functioning 'normally'. The toilet model investigated was a standard wash-down design, which is present in many patient areas of the hospital. Some newer hospital areas have alternative designs. National Health Service guidance (SHTM 64 Sanitary Assemblies; www.spaceforhealth.nhs.uk) recommends installation of toilets with a hospital pattern rimless pan and a raised seat and that are water saving (Health Facilities, Note 30; www.spaceforhealth.nhs.uk). Actually, our assessment of toilets in clinical areas showed that such models produced more droplets than the standard wash-down design. It is not known whether the findings from this study would be applicable to newer toilet styles, such as those that use less water. The toilet area used was not ventilated (no extractor fan and the window was closed) and it is possible that such options would help to remove airborne bacteria. However, as the particle deposition occurred rapidly following flushing, it is doubtful that a high proportion of such droplets would be effectively removed by toilet ventilation, which is designed primarily to remove odours.

The majority of toilets installed in hospital patient areas are not fitted with lids, as is also the case in many public areas. The reasoning used is that lids may be a source of bacteria, are not desirable to touch, and may therefore not be used. It has also been suggested that lids may make it harder to clean toilets, as they are another surface to decontaminate. However, discussion with cleaning staff suggests that the latter is not a major issue, and a method is in place to clean lidded toilets. Our findings have implications for infection prevention and control practices, notably in hospital environments where lidless toilets are present and are likely to be used by patients, including those with infective and non-infective diarrhoea. Shared toilets are commonplace within a hospital environment and our data suggest that lidless toilets could be a vehicle for *C. difficile*

contamination and thus transmission of bacteria. Although patients with known CDI should use either a dedicated toilet or commode, this may not happen early in the course of symptoms, and because patients may prefer to use shared toilets in preference to a commode. Other patients may also excrete high numbers of *C. difficile* (asymptomatic carriers, especially those with diarrhoea due to other causes) and may be an inadvertent source of toilet environment contamination. We speculate that such contamination could permit transmission of *C. difficile* from asymptomatic carriers, and thus explain some CDI cases where no apparent linked CDI cases are found.[14,15]

The scope for environmental seeding associated with toilet flushing highlights the imperative for hand washing after toilet use, and frequent cleaning to remove contamination. Toilets with improved design that do not create aerosols are desirable. Our results demonstrate that if lids are fitted to current models they will very likely become contaminated upon flushing. It is already known that *C. difficile* may spread markedly in hospitals. Lidless conventional toilets increase the risk of *C. difficile* environmental contamination, and thus we suggest that their use is discouraged, particularly in settings where CDI is common.

Conflict of interest statement
None declared.

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