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Original Article

Bioaerosols generated from toilet flushing in rooms of patients with *Clostridioides difficile* infection

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Abstract

Background: *Clostridioides difficile* infection (CDI) is the most frequently reported hospital-acquired infection in the United States. Bioaerosols generated during toilet flushing are a possible mechanism for the spread of this pathogen in clinical settings.

Objective: To measure the bioaerosol concentration from toilets of patients with CDI before and after flushing.

Design: In this pilot study, bioaerosols were collected 0.15 m, 0.5 m, and 1.0 m from the rims of the toilets in the bathrooms of hospitalized patients with CDI. Inhibitory, selective media were used to detect *C. difficile* and other facultative anaerobes. Room air was collected continuously for 20 minutes with a bioaerosol sampler before and after toilet flushing. Wilcoxon rank-sum tests were used to assess the difference in bioaerosol production before and after flushing.

Setting: Rooms of patients with CDI at University of Iowa Hospitals and Clinics.

Results: Bacteria were positively cultured from 8 of 24 rooms (33%). In total, 72 preflush and 72 postflush samples were collected; 9 of the preflush samples (13%) and 19 of the postflush samples (26%) were culture positive for healthcare-associated bacteria. The predominant species cultured were *Enterococcus faecalis*, *E. faecium*, and *C. difficile*. Compared to the preflush samples, the postflush samples showed significant increases in the concentrations of the 2 large particle-size categories: 5.0 μm ($P = .0095$) and 10.0 μm ($P = .0082$).

Conclusions: Bioaerosols produced by toilet flushing potentially contribute to hospital environmental contamination. Prevention measures (eg, toilet lids) should be evaluated as interventions to prevent toilet-associated environmental contamination in clinical settings.

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Bioaerosols are a major environmental contaminant in healthcare settings, and flushing uncovered toilets is a potential source of pathogenic bioaerosols like *Clostridioides difficile*.[1–4] *Clostridioides* (formerly *Clostridium*) *difficile* is a gram-positive, anaerobic, spore-forming bacterium shed from the gastrointestinal tract,[5–7] and *C. difficile* infection (CDI) is the most commonly reported hospital-acquired infection in the United States.[8] It affects nearly half a million people and causes ~15,000 deaths annually.[9] The spore-forming nature of *C. difficile* allows the bacteria to persist in the environment. *Clostridioides difficile* spores can contaminate a hospital room; they have been found on toilets, bedsheets, bedpans, wash basins, bed rails, floors, and walls of rooms occupied by patients with CDI.[10–13] Several studies have experimentally demonstrated the production of aerosolized *C. difficile* spores from toilet plumes.[3,4,8,14]

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Intestinal dysbiosis permits colonization and infection with *C. difficile*, which could lead to an increase in other intestinal pathogenic organisms such as *Enterococcus*, another serious hospital-acquired infection.

Enterococcus is a genus of gram-positive cocci-shaped bacteria. *Enterococci* are facultative anaerobes, meaning they can survive in oxygen-poor environments.[15] Two species of *Enterococcus* (*E. faecalis* and *E. faecium*) are commensal bacteria found in >90% of human intestinal microbiomes. However, these strains can be pathogenic due to overproliferation via intestinal dysbiosis. Additionally, these strains can become vancomycin resistant by acquiring β-lactam–resistant genes.[15,16] Vancomycin-resistant *Enterococcus* (VRE) infections account for ~4% of all reported hospital-acquired infections in the United States.[17] A large study of 1,647 environmental samples collected in a hospital reported a 12% positive culture rate for VRE infected patients' rooms had a culture positive rate of 70%.[18]

A previous study conducted by at the University of Iowa quantifying bioaerosols produced from a toilet in a healthcare setting

found that the concentration of bioaerosols from hospital bathrooms was 210 colony-forming units per cubic meter (CFU/m^3) at baseline.[19] In the current study, we aimed to determine the extent to which toilet flushing increases bioaerosol contamination in bathrooms of patients with CDI.

Methods

Room notifications and patient consent

All trials were conducted in the following units at the University of Iowa Hospitals and Clinics (UIHC): neurology, surgery specialty, gynecology/oncology, urology, surgery/hematology, medical observation, medical specialty, or bone marrow transplant. UIHC infection preventionists notified the research team of admitted patients who had been diagnosed with CDI within the previous 24 hours. The bathrooms of patients with CDI were sampled within 48 hours of patient diagnosis. If the patient had been discharged and terminal cleaning of the room had already been completed, sampling was not performed.

All patients were given a brief explanation of the goal of the study and were asked for verbal consent to perform sampling. If consent was not given, the room was not sampled. There was no requirement for waste to be visible in the toilet because previous studies have shown that toilets that appear empty produce detectable levels of aerosolized bacteria. All sampling was completed between December 2017 and September 2018. The goal of this pilot study was to collect samples from 25 rooms. This study was approved by University of Iowa Institutional Review Board (IRB no. 201601705).

Bioaerosol sampling description

Bioaerosol sampling was completed using a specially designed bioaerosol sampling cart used in previous bioaerosol research (Supplemental Fig. 1 online).[19] The cart comprised 3 bioaerosol samplers (BioStage impactors, SKC, Eighty Four, PA) attached to the side of the cart so that when the leading edge was flush with the toilet rim, the samplers were at predetermined distances of (0.15 m, 0.5 m, and 1.0 m). The samplers were 0.6 m from the floor and 0.23 m above the rim of toilet. Each sampler was connected to an encased pump (serial nos. VP0935A-V1028-D2-0511; 00601036, AC0401A-A1110-E1-178; and I1001004T, I1001005T; Medo, Roselle, IL). The airflow for each pump was adjusted independently via a flow control valve (0.95-cm 2-way valve; serial no. 104, 104-N03, Ingersoll Rand, Davidson, NC). A primary standard airflow calibration device (Bios Defender 510, Mesa Labs, Butler, NJ) was used calibrate all sampling to a flow rate of 28.3 L/min before sampling began.

Sample collection and incubation

The sampling cart was placed directly against the toilet. An optical particle counter (AeroTrak particle counter 9306-V, TSI, Shoreview, MN) was placed on a level surface ~18 cm from the toilet rim and between 12 cm and 18 cm from the ground. Particle concentrations were measured at 6 different sizes: 0.3 μm, 0.5 μm, 1.0 μm, 3.0 μm, 5.0 μm, and 10.0 μm. Data were recorded every 60 seconds during sampling. Particle concentrations were measured for 19 of the 24 trials. Particle concentration data were summed for each minute of the trial, and recording continued for 40 minutes.

Pre-reduced cycloserine-cefoxitin fructose agar with horse blood and taurocholate (CCFA-HT) plates, a selective media for *C. difficile* anaerobic growth, were placed into each sampler, with an additional covered plate placed on top of the cart as a negative control. All equipment was properly placed before flush sampling began. Air was sampled continuously for 20 minutes. During the sampling, the researcher detailed all activity in the patient room. The door separating the bathroom from the patient room was open during sampling, so although the primary source of aerosols was the bathroom, a small amount of room air was also potentially sampled.

After preflush sampling was completed, all equipment was turned off, and preflush plates were removed from the sampler, labeled, and placed in a cooler for transport to the lab. New CCFA-HT plates were placed into each sampler, and all equipment was activated again. After 3 minutes of sampling, the toilet was flushed, and sampling continued for an additional 20 minutes. There was a total of 8 plates were collected during each trial: 1 at each distance before flushing, 1 at each distance after flushing, as well as 1 control plate before flushing and 1 control plate after flushing. The dimensions of the room and the humidity levels were also recorded. A sling psychrometer (Bacharach, Pittsburgh, PA) was used to measure the relative humidity in the room.

Bioaerosol content culture and identification

Plates were incubated in an anaerobic chamber for 48 hours after sample collection. Total colony counts were obtained at 24 and 48 hours after sampling. Each distinct colony phenotype was transferred onto a separate CCFA-HT plate and incubated for an additional 48 hours in the anaerobic chamber. The genus and species of each colony were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The primary organism of interest was *C. difficile*; therefore, a highly selective media and anaerobic incubation method was used to promote *C. difficile* growth. Aerobic organisms or organisms not suited to grow in this environment would not have been detected and are underrepresented in this study.

Statistical analysis

Bacterial density was measured as colony-forming units per cubic meter of air sampled (CFU/m^3). This value was calculated by multiplying air flow rate by the number of minutes sampled to determine the total volume of air sampled at that trial. The number of colonies present after 48 hours incubation was then divided by the total air volume sampled, and that value was multiplied by 1,000. A trial was counted as positive if at least 1 of the 6 plates used during that sampling time showed at least 1 colony of bacterial growth after incubation.

To account for pairing between preflush and postflush samples, the McNemar test was used to compare the rate of positive cultures, and the Wilcoxon signed-rank test was used to compare particle concentration and bacterial density.

Results

In total, 24 bathrooms were sampled before and after flushing, and 8 of these bathrooms yielded growth on at least 1 of the 6 plates collected from each bathroom during aerosol sampling, which we defined as a positive sample. For the remaining 16 bathrooms, no growth was detected on any of the sampling plates (Table 1). We detected no difference between rooms that had positive culture and rooms that had negative culture by hospital unit, presence of waste in the toilet, or amount of time from notification to room

Table 1. Demographics of Patient Rooms

Variable	Rooms With Positive Cultures (n = 8), No. (%)	Rooms With Negative Cultures (n = 16), No. (%)	*P* Value[a]
Unit			
Hematology/Oncology	3 (38)	2 (13)	.9347
Medical specialty	2 (25)	6 (38)	
Bone marrow transplant	2 (25)	3 (19)	
Oncology	0	2 (13)	
Surgical specialty	0	1 (6)	
Orthopedics/Urology	1 (13)	2 (13)	
Waste in toilet			.9207
No	6 (75)	12 (75)	
Yes (urine or stool)	2 (25)	3 (19)	
Unknown	0	1 (6)	
Days since CDI diagnosis, median (IQR)	1.5 (0.0–3.0)	1.5 (0.0–3.0)	.8183
Patient room relative humidity, median (IQR)	71% (60%–82%)	68% (47%–82%)	.2562

Note. CDI, *Clostridioides difficile* infection; IQR, interquartile range.
[a]The Fisher exact test was used for categorical variables, and the Wilcoxon rank-sum test was used for continuous variables.

Table 2. Positive Bioaerosol Cultures

			Preflush[a]			Postflush[a]		
Trial	Waste in Toilet	Bacteria	0.15 m	0.50 m	1.00 m	0.15 m	0.50 m	1.00 m
2	No	*Enterococcus faecium*	0	0	0	**25.1**[b]	**23.7**	**49.0**
		E. faecalis	0	0	0	**16.2**	**10.4**	0
		Unidentified	0	0	0	0	0	**2.9**
4	No	*C. difficile*	**7.1**	**10.6**	**17.7**	**7.4**	**4.4**	**5.9**
6	No	*E. faecium*	0	**1.8**	0	0	0	0
14	Urine	*B. thuringenisis*	0	0	**1.8**	0	0	0
17	Stool	*C. difficile*	0	0	0	**4.6**	**5.9**	**5.9**
19	No	*E. faecalis*	**7.2**	**5.3**	0	0	**4.4**	**5.9**
		Unidentified	0	0	**7.1**	**7.6**	0	0
20	No	*E. faecium*	0	0	0	**1.5**	0	0
22	No	*E. faecalis*	0	0	**1.8**	**3.3**	**2.9**	**4.4**

Note. CFU, colony-forming units.
[a]Values represent the bioaerosol concentration given in CFU/m^3 for each organism cultured.
[b]Values above zero are shown in bold.

sampling. The average relative humidity was higher in rooms that yielded a positive culture than in rooms that yielded a negative culture, although this difference was not statistically significant. The mean average relative humidity in positive-culture rooms was 72% (interquartile range [IQR], 60%–82%) versus 68% (IQR, 47%–82%) in negative-culture rooms.

The culture-positive trials are shown in Table 2. Of these 8 trials, 6 were performed on toilets with no visible waste; 1 trial was performed on a toilet containing urine; and 1 trial was performed on a toilet containing stool. Only 1 trial showed a positive culture at all 3 distances both before and after flushing, and 4 additional trials showed a positive culture on at least 3 of the 6 sample plates used. The remaining 3 trials showed bacterial growth on only 1 of the 6 sample plates. In total, 28 bacterial identifications were made from the 8 culture-positive rooms. The most frequently cultured species was *Enterococcus faecalis* (36%), followed by *C. difficile* (32%), and *Enterococcus faecium* (18%). Environmental bacilli accounted for 3% of the species found, and 11% of the colonies grown could not be identified via MALDI-TOF.

To determine whether aerosolization from the toilet plume was affecting the bioaerosol distribution, the culture rates were compared using samples taken before and after flushing. The percentage of positive preflush plates was 12.5% (9 of 72), and the percentage of positive postflush plates was 26.4% (19 of 72). Postflush plates had a significantly higher probability of culturing positive than preflush plates ($P = .0309$).

At 0.15 m from the edge of the toilet, the mean bioaerosol concentration before flushing was 1.3 CFU/m^3 compared to 5.9 CFU/m^3 after flushing. The mean concentration before flushing at 0.5 m was 1.6 CFU/m^3 compared to 4.7 CFU/m^3 after flushing. Finally,

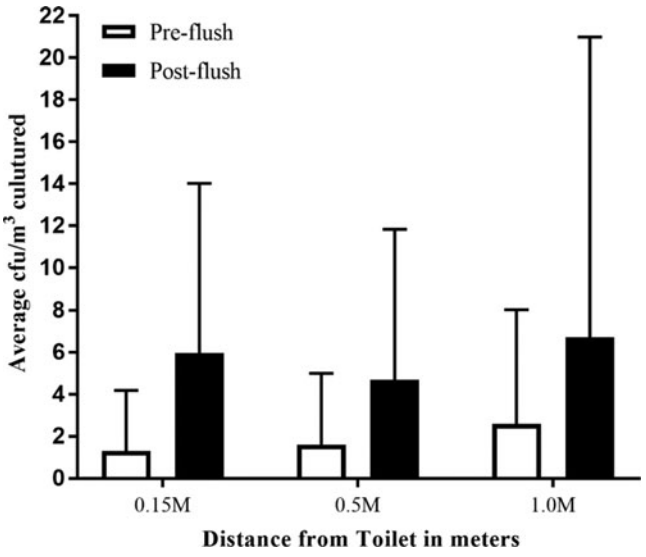

Fig. 1. Bioaerosol by distance before and after flushing. Mean and standard deviations are shown. Results of the total colony count are from the original sample plate and represent both pathogenic and apathogenic bacteria.

the 1.0-m concentration before flushing was 2.6 CFU/m^3 compared to 6.7 CFU/m^3 after flushing. Although we detected no significant differences, concentrations were higher after flushing at all 3 distances, and the distance closest to the toilet (0.15 m) trended toward significance ($P = .0781$) (Fig. 1).

Across all trials, we detected a significant increase in particle concentrations after flushing compared to before flushing at the 2 largest particle sizes measured: 5.0 μm ($P = .0095$) and 10.0 μm ($P = .0082$). Concentrations of the smallest and the 2 largest particle sizes (0.3 μm, 5.0 μm, and 10.0 μm) significantly increased after flushing in the positive culture trials. No difference was detected in concentrations of particles from negative-culture trials (Fig. 2).

Discussion

Toilet plumes are a potential source of pathogenic bioaerosol contamination in patient rooms.[1,14,20] In this study, we evaluated the bioaerosol production and particle concentration from the toilet plumes of toilets in rooms of patients with CDI. We collected 24 before-and-after samples in patient bathrooms during this pilot study. We found a 2-fold increase of positive cultures in postflush samples compared to preflush samples. In addition, the likelihood of culturing positive was affected by distance both before and after flushing. However, our pilot study was underpowered to detect a true difference before versus after flushing. Furthermore, bacteria were only detected in one-third of the sampled rooms. Most bacteria identified were pathogenic (86%); however, this result was most likely an overestimation due to our culturing methods. We detected an increase in bioaerosols after flushing compared to baseline, which suggests that the act of flushing the toilet could impact the amount of bioaerosols in the room.

An increase in particle concentration was strongly associated with positive bioaerosol culture, indicating an overall higher level of contamination in the air after flushing in those rooms. Humidity might have utility as an indicator of a positive culture because the rooms with a positive culture were, on average, 3% more humid than negative-culture rooms. Thus, humidity might play a factor in the concentration and dispersal patterns of bioaerosols in the environment.

Enterococcus spp. represented 54% of the total positive cultures. This finding is notable because *Enterococcus* is another major hospital-associated infection in which hospital environmental contamination can play a significant role in patient transmission.[18] Although resistance testing was not performed on these samples, the presence of *Enterococcus* should be evaluated further because the presence of VRE aerosolized samples could significantly contribute to VRE environmental contamination.

The results from this pilot study echo findings from other studies in which *C. difficile* was cultured from hospital toilets. Roberts et al[20] used a portable cyclone sampler for 4 days over a 2-year period in 2 separate wards to test for evidence of *C. difficile* contamination, and *C. difficile* spores were detected in 50% of the trials. Although we only detected bacteria in 19% of total number of samples collected in our study, *C. difficile* was detected in 32% of those samples, comparable to the study by Roberts et al.

Best et al[4] experimentally seeded toilets with 10^7 CFU/mL of *C. difficile*, a concentration that is comparable to the amount of bacteria shed from an infected person's stool. They performed flush experiments to determine the number of *C. difficile* containing bioaerosols created by the toilet.[4] An air sampler continuously collected measurements for 90 minutes after the toilet was flushed. The *C. difficile* aerosolized samples measured 36 CFU immediately after the toilet was flushed. In our present study, we improved upon these previous designs by sampling bioaerosols from toilets in the rooms of patients with active *C. difficile* infections. The previous studies were conducted in controlled environments under conditions that are not consistent with the healthcare setting.

The strengths of this pilot study included a detailed analysis of factors that could impact aerosol production in the room. Additionally, the collection of preflush samples provided a baseline concentration of bioaerosols in the air, thus increasing the likelihood that the observed postflush bioaerosol concentrations were attributable to the toilet plume. Previous studies have demonstrated that bioaerosols can be found in high concentrations in a patient bathrooms. However, previous research only identified total aerosolized bacteria and not specific pathogens, as we did in this study.[19] This work builds on previous studies by quantifying and defining the bacterial bioaerosols captured.

This study has several limitations. First, it was a pilot study with a limited sample size. Several rooms were not sampled because patients were reluctant to lose access to their bathrooms for the 60-minute trial. Because CDI promotes frequent diarrhea, not including patients with potentially more severe symptoms may have excluded the more heavily contaminated toilets. Additionally, the sample collection protocol used was time-consuming, which limited the number of rooms that could be sampled per day. The plates used for culturing were designed to detect *C. difficile* and were incubated in an anaerobic chamber; therefore, culture and detection of many aerobic pathogenic bacteria, such as *Escherichia coli*, was not possible.

To account for these limitations, future studies should consider using plates and culture methods designed to detect a broader range of bacteria. Also, the room of patients infected with other gastrointestinal pathogens, as well as the rooms of healthier control patients, should be cultured for a better understanding of normal bioaerosols in hospitals and not just those of patients with CDI. Finally, the use of a smaller, higher flow rate sampling device would allow for a shorter sampling time and less labor-intensive process, which would allow additional rooms to be sampled during any given period.

In conclusion, toilet flushes are a potential source of bioaerosols that could lead to environmental contamination and infection

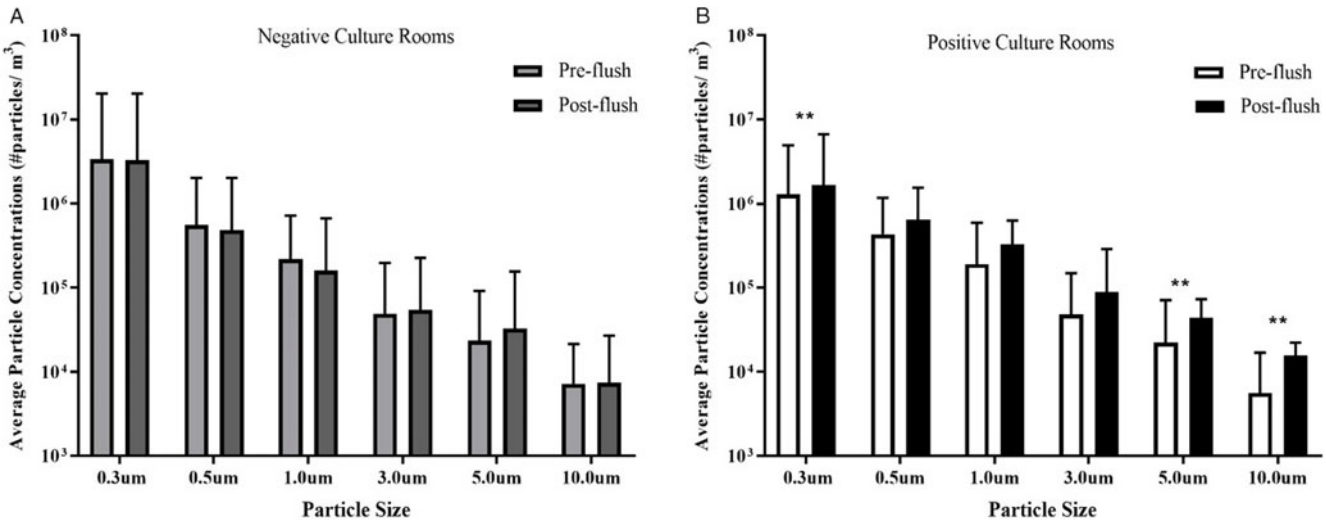

Fig. 2. Preflush and postflush particle concentration data are shown for both negative rooms (n = 13) and positive rooms (n = 6) where complete data were obtained. The average particle concentration did not significantly change in any negative culture rooms. The particle concentration significantly increased (**$P > .05$) for the 0.3-μm, 5.0-μm, and 10-μm particle sizes in the positive-culture rooms.

transmission in the healthcare setting. Patients who are infected with enteric pathogens require frequent toilet usage, which leads to heavy toilet contamination. This study potentially supports the hypothesis that toilet flushing may lead to the spread of clinically significant pathogens in healthcare settings. More information is needed to determine the risk factors associated with toilet flushing and environmental contamination by pathogens.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/ice.2020.11

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References

1. Johnson DL, Mead KR, Lynch RA, Hirst DV. Lifting the lid on toilet plume aerosol: a literature review with suggestions for future research. *Am J Infect Control* 2013;41:254–258.
2. Zemouri C, de Soet H, Crielaard W, Laheij A. A scoping review on bioaerosols in healthcare and the dental environment. *PloS One* 2017;12:e0178007.
3. Best EL, Fawley WN, Parnell P, Wilcox MH. The potential for airborne dispersal of *Clostridium difficile* from symptomatic patients. *Clin Infect Dis* 2010;50:1450–1457.
4. Best EL, Sandoe JA, Wilcox MH. Potential for aerosolization of *Clostridium difficile* after flushing toilets: the role of toilet lids in reducing environmental contamination risk. *J Hosp Infect* 2012;80:1–5.
5. Ghose C. *Clostridium difficile* infection in the twenty-first century. *Emerg Microbes Infect* 2013;2:e62.
6. Janezic S, Rupnik M. Genomic diversity of *Clostridium difficile* strains. *Res Microbiol* 2015;166:353–360.
7. O'Neill G, Adams JE, Bowman RA, Riley TV. A molecular characterization of *Clostridium difficile* isolates from humans, animals and their environments. *Epidemiol Infect* 1993;111:257–264.
8. Magill SS, O'Leary E, Janelle SJ, *et al.* Changes in prevalence of healthcare-associated infections in US hospitals. *N Engl J Med* 2018;379:1732–1744.
9. Nearly half a million Americans suffered from *Clostridium difficile* infections in a single year. Centers for Disease Control and Prevention website. https://www.cdc.gov/media/releases/2015/p0225-clostridium-difficile.html. Published 2015. Accessed January 14, 2020.
10. Fekety R, Kim KH, Brown D, Batts DH, Cudmore M, Silva J Jr. Epidemiology of antibiotic-associated colitis; isolation of *Clostridium difficile* from the hospital environment. *Am J Med* 1981;70:906–908.
11. Kim KH, Fekety R, Batts DH, *et al.* Isolation of *Clostridium difficile* from the environment and contacts of patients with antibiotic-associated colitis. *J Infect Dis* 1981;143:42–50.
12. 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.
13. Weinstein RA, Hota B. Contamination, disinfection, and cross-colonization: are hospital surfaces reservoirs for nosocomial infection? *Clin Infect Dis* 2004;39:1182–1189.
14. Barker J, Jones MV. The potential spread of infection caused by aerosol contamination of surfaces after flushing a domestic toilet. *J Appl Microbiol* 2005;99:339–347.
15. Fisher K, Phillips C. The ecology, epidemiology and virulence of *Enterococcus*. *Microbiology (Reading, England)* 2009;155:1749–1757.
16. Satlin MJ, Walsh TJ. Multidrug-resistant Enterobacteriaceae, *Pseudomonas aeruginosa*, and vancomycin-resistant *Enterococcus*: three major threats to hematopoietic stem cell transplant recipients. *Transplant Infect Dis* 2017;19(6). doi: 10.1111/tid.12762.
17. Hidron AI, Edwards JR, Patel J, *et al.* NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infect Control Hosp Epidemiol* 2008;29:996–1011.
18. McDermott H, Skally M, O'Rourke J, Humphreys H, Fitzgerald-Hughes D. Vancomycin-resistant enterococci (VRE) in the intensive care unit in a non-outbreak setting: identification of potential reservoirs and epidemiological associations between patient and environmental VRE. *Infect Control Hosp Epidemiol* 2018;39:40–45.
19. Knowlton SD, Boles CL, Perencevich EN, *et al.* Bioaerosol concentrations generated from toilet flushing in a hospital-based patient care setting. *Antimicrob Resist Infect Control* 2018;7:16.
20. Roberts K, Smith CF, Snelling AM, *et al.* Aerial dissemination of *Clostridium difficile* spores. *BMC Infect Dis* 2008;8:7.