Open Forum Infectious Diseases

MAJOR ARTICLE

IDSA Infectious Diseases Society of America hivma hiv medicine association OXFORD

The Impact of Infection Versus Colonization on *Clostridioides difficile* Environmental Contamination in Hospitalized Patients With Diarrhea

Bobby G. Warren,[1,2] Nicholas A. Turner,[1,2] Rachel Addison,[1,2] Alicia Nelson,[1,2] Aaron Barrett,[1,2] Bechtler Addison,[1,2] Amanda Graves,[1,2] Becky Smith,[1,2] Sarah S. Lewis,[1,2] David J. Weber,[3] Emily E. Sickbert-Bennett,[3] Deverick J. Anderson[1,2]; and the CDC Prevention Epicenters Program

[1]Duke Center for Antimicrobial Stewardship and Infection Prevention, Durham, North Carolina, USA, [2]Division of Infectious Diseases, Duke University Medical Center, Durham, North Carolina, USA, [3]Division of Infectious Diseases, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Background. Patients with *Clostridioides difficile* infections (CDIs) contaminate the healthcare environment; however, the relative contribution of contamination by colonized individuals is unknown. Current guidelines do not recommend the use of contact precautions for asymptomatic *C difficile* carriers. We evaluated *C difficile* environmental contamination in rooms housing adult inpatients with diarrhea based on *C difficile* status.

Methods. We performed a prospective cohort study of inpatient adults with diarrhea who underwent testing for CDI via polymerase chain reaction (PCR) and enzyme immunoassay (EIA). Patients were stratified into cohorts based on test result: infected (PCR^+/EIA^+), colonized (PCR^+/EIA^-), or negative/control (PCR^-). Environmental microbiological samples were taken within 24 hours of *C difficile* testing and again for 2 successive days. Samples were obtained from the patient, bathroom, and care areas.

Results. We enrolled 94 patients between November 2019 and June 2021. *Clostridioides difficile* was recovered in 93 (38%) patient rooms: 44 (62%) infected patient rooms, 35 (43%) colonized patient rooms (P = .08 vs infected 38 patient rooms), and 14 (15%) negative patient rooms (P < .01 vs infected; P < .01 vs colonized). *Clostridioides difficile* was recovered in 40 (56%), 6 (9%), and 20 (28%) of bathrooms, care areas and patient areas in 40 infected patient rooms; 34 (41%), 1 (1%), and 4 (5%) samples in colonized patient rooms; and 12 (13%), 1 (1%), and 3 (3%) of samples in negative patient rooms, respectively.

Conclusions. Patients colonized with *C difficile* frequently contaminated the hospital environment. Our data support the use of contact precautions when entering rooms of patients colonized with *C difficile*, especially when entering the bathroom.

Keywords. *Clostridioides difficile*; contact precautions; environment; infection prevention.

Clostridioides difficile infections (CDIs) are a frequent cause of healthcare-associated infections in the United States. Approximately 500 000 CDI occur each year, leading to approximately 29 000 deaths [1, 2]. Infection prevention teams deploy many techniques to prevent CDI in healthcare settings including hand hygiene, environmental disinfection, and contact precautions. However, United States incidence rates have only minimally decreased [3].

Infected individuals shed *C difficile* spores, which leads to contamination of the hospital environment and increased risk of transmission [4]. However, the contribution of colonized individuals to environmental contamination and risk of transmission is largely unknown [2, 5]. Published guidelines do not recommend the use of contact precautions for asymptomatic *C difficile* carriers or offer no recommendation due to the lack of data [6–8]. Our study aimed to address this lack of data.

We conducted a prospective cohort study to measure the relative contributions of infected, colonized, or control (*C difficile* negative) patients to *C difficile* environmental contamination. We hypothesized that rooms housing patients with CDI would have higher *C difficile* recovery rates than rooms with either patients with *C difficile* colonization or control patients.

METHODS

Study Setting and Design

We performed a prospective cohort study of inpatient adults with diarrhea who were tested for *C difficile* infection via polymerase chain reaction (PCR) and enzyme immunoassay (EIA) at Duke University Health System (DUHS) in Durham, North Carolina. Our primary objective was to evaluate *C difficile* environmental contamination among adult inpatients with diarrhea based on *C difficile* status. Patients were stratified into 1 of

Received 29 November 2021; editorial decision 2 February 2022; accepted 7 February 2022; published online 9 February 2022.

Correspondence: Bobby G. Warren, MPS, 325 Trent Drive Durham, NC 27710, USA (bobby.warren@duke.edu).

Open Forum Infectious Diseases®2022

© The Author(s) 2022. Published by Oxford University Press on behalf of Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

https://doi.org/10.1093/ofid/ofac069

3 groups based on test results and based on previous data suggesting validity of test results indicating true disease: infected (PCR^+/EIA^+), colonized (PCR^+/EIA^-), or control (PCR^-) [9]. Patients were excluded if they had a positive *C difficile* PCR or EIA in the prior 6 weeks. All PCR and EIA assays were performed by the DUHS clinical microbiology laboratory. Polymerase chain reaction assays were completed on all suspected *C difficile* infections, and EIA assays (sensitivity, 87.8%; specificity, 99.4%) were only completed if the clinical specimen was PCR^+. Both PCR and EIA results were reported to clinicians.

Patient Consent Statement

This study was designated as exempt from institutional review board review by the Duke University Health System Institutional Review Board.

Study Procedures

Routine disinfection was performed in all study rooms per standard hospital protocols, and all rooms were single patient rooms with no shared bathrooms. For a room housing a patient with CDI colonization or infection, Environmental Services (EVS) performed daily disinfection with bleach, excluding floors, and terminal disinfection using bleach and UV-C. For other rooms, EVS used bleach for both terminal and daily disinfection. The EVS team was blinded to study assignment or study activities during the study.

Environmental microbiological samples were taken for a total of 3 days: within 24 hours of *C difficile* testing and again for 2 successive days. Samples were obtained from 3 locations within each study room: patient area, bathroom area, and care area. The patient area included the patient bedrails and the surface of the overbed table, totaling approximately 3000 cm^2. The bathroom area included the top of the bathroom sink's bowl, the toilet seat, and the floor around the base of the toilet (1750 cm^2). The care area included the clinician's in-room computer keyboard, computer mouse, and intravenous poles adjacent to the patient bed (1450 cm^2). Sampling protocols were used to ensure the same locations and surface area were cultured each time.

Microbiological Methods

All cultures were obtained directly from the clinical environment with premoistened cellulose sponges using the sponge and stomacher technique per the Centers for Disease Control and Prevention protocol [10]. Sponges were placed in stomacher bags with 45 mL phosphate-buffered saline with 1% Tween 20 and homogenized for 60 seconds at 260 revolutions per minute (RPM). Homogenates were then centrifuged at 3200 RPM for 15 minutes, and all but approximately 5 mL of the resulting supernatant was discarded. Then, each sample was rehomogenized via vortex. A total of 200 µL of the final homogenate was plated onto *C difficile* selective agar and incubated anaerobically at 37°C for 48 hours. Species was confirmed using standard laboratory procedures.

Ribotyping

Polymerase chain reaction ribotyping was completed to measure concordance of isolates using previously published primers [11]. Deoxyribonucleic acid extractions were completed by suspending 10 colonies of isolated *C difficile* per sample, to assess for presence of more than 1 strain, in a 100-µL, 5% Chelex solution and incubated at 100°C for 10 minutes. Samples were then centrifuged at 13 000 RPM for 10 minutes, and supernatants were isolated and used as PCR templates. The PCR conditions were as follows: initial denaturation of 5 minutes at 95°C, followed by 35 cycles of 1 minute at 95°C (denaturization), 1 minute at 57°C (annealing), and 1 minute at 72°C (elongation), followed by 10 minutes at 72°C (final elongation) and 45 minutes at 75°C to concentrate amplification products. Amplified PCR products underwent gel electrophoresis in a 3% agarose gel for 16 hours at 50 volts with chilled 1× TBE. Patient and environmental isolates were assessed for concordance using the number of banding pattern differences previously described [12].

Outcomes and Data Analysis

Study data were summarized using descriptive statistics, including means (±standard deviation) and medians (interquartile range [IQR]), as appropriate. The primary study outcomes were colony-forming units (CFUs) and recovery rates between arms, compared with a global analysis of variance followed by pairwise comparisons using a Bonferroni adjustment. $P < .05$ was considered significant; all statistical tests were 2-tailed and were performed using R software (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

We enrolled 94 patients between November 2019 and June 2021; 28 were infected, 32 were colonized, and 34 were controls. Overall, 47 (50%) were female and the median age of the entire cohort was 65 years (IQR, 55–72). In general, the patients in each group were similar, although patients in the control arm were less often on contact precautions, which was expected (Table 1). A total of 740 individual samples were obtained during 247 room microbiological sampling sessions.

In general, the highest burden of *C difficile* contamination was seen in infected patient rooms (Table 2). The mean room CFU was 337 (±913) in infected patient rooms, 221 (±811) in colonized patient rooms, and 94 (±637) in control patient rooms ($P = .14$) (Table 2). Overall, *C difficile* was recovered in 93 (38%) sampling sessions. Compared to controls, *C difficile* was recovered more frequently in infected ($P < .01$) and colonized rooms ($P < .01$) (Table 3, Figure 1). However, the frequency of recovery was not statistically different between

Table 1. Patient Characteristics

Characteristics	Total (%) N = 94	Infected N = 28 n (%)	Colonized N = 32 n (%)	Control N = 34 n (%)
Median age, years (IQR)	65 (55–72)	68 (60–74)	65 (53–74)	62 (52–71)
Female sex	47 (50)	12 (43)	19 (59)	16 (47)
On contact precautions	69 (73)	28 (100)	31 (97)	10 (35)
Bedridden	31 (33)	10 (36)	9 (28)	12 (35)
Average bowel movements with 24 hours of enrollment (SD)	4 (3)	5 (2)	4 (3)	4 (4)
Prior room occupant *Clostridioides difficile* positive	3 (3)	2 (7)	0 (0)	1 (1)
Hospitalized in last 12 months	67 (71)	21 (75)	19 (59)	27 (79)
Antibiotic therapy in prior 6 months	49 (52)	16 (57)	16 (50)	17 (50)
Antibiotic therapy in prior 24 hours	67 (71)	22 (79)	21 (66)	24 (71)
Median number of days patient was in the room before sampling (IQR)	2 (1–8)	2 (0–8)	2 (1–9)	3 (1–8)

Abbreviations: IQR, interquartile range; SD, standard deviation.

infected and colonized rooms (P = .08). These analyses were repeated excluding day 3 because some patients were lost to discharge or moving rooms and the results remained the same.

In general, room contamination peaked on the second day of collection in rooms with PCR$^+$ patients (Figure 2). The mean room CFU was 96 (±296), 167 (±832), and 63 (±197) on sample days 1, 2, and 3, in infected patient rooms and 36 (±128) 147 (±722), and 24 (±133) in colonized patient rooms, respectively (Table 2). In contrast, the mean CFU was 66 (±604), 1 (9), and 23 (130) in control patient rooms on sample days 1, 2, and 3. *Clostridioides difficile* was recovered in the following: 26 (31%), 23 (31%), and 17 (31%) of infected patient rooms on sample days 1, 2, and 3; 16 (17%), 17 (19%), and 6 (10%) in colonized patient rooms on sample days 1, 2, and 3; and 6 (6%), 3 (3%), and 7 (8%) in control patient rooms on sample days 1, 2, and 3 (Table 3).

With regard to sample location, the mean bathroom, care area, and patient area CFU were 303 (±890), 4 (±13), and 30 (±102) in infected patient rooms, 218 (±811), 1 (±3), and 2 (±13) in colonized patient rooms, and 93 (±640), 1 (±3), and 1 (±6) in control patient rooms (Table 2). Overall, *C difficile* was recovered in 86 (35%) of bathroom samples, 8 (3%) of care area samples, and 27 (11%) of patient area samples. At the study arm level, *C difficile* was recovered in the following: 40 (56%), 6 (9%), and 20 (28%) of infected patients' bathroom, care area, and patient area samples; 34 (41%), 1 (1%), and 4 (5%) of colonized patients' bathroom, care area, and patient area samples; and 12 (13%), 1 (1%), and 3 (3%) of control patients' bathroom, care area, and patient area samples (Table 3).

Ribotyping data were available on 86 isolates (25 patient and 61 environment). Eighty-one percent of patient rooms with available patient and environmental isolates had a concordant isolate recovered in the environment; 15% of

Table 2. Mean (±SD) *Clostridioides difficile* CFUs by Study Arm, Study Day, and Sample Location

Sample Day	Sample Area	Infected CFU (SD)	Colonized CFU (SD)	Control CFU (SD)	*P* Infected vs Control, Colonized vs Control, Infected vs Colonized
Total	Room	337 (913)	221 (811)	94 (637)	.14
	Bathroom	303 (890)	218 (810)	93 (640)	
	Care area	4 (13)	1 (3)	1 (3)	
	Patient area	30 (102)	2 (13)	1 (6)	
Sample Day 1	Room	96 (296)	36 (128)	66 (604)	
	Bathroom	232 (463)	102 (207)	197 (1045)	
	Care area	4 (16)	1 (5)	0 (0)	
	Patient area	50 (157)	5 (20)	0 (0)	
Sample Day 2	Room	167 (832)	147 (772)	1 (9)	
	Bathroom	481 (1407)	493 (1303)	3 (15)	
	Care area	1 (6)	0 (0)	0 (0)	
	Patient area	18 (40)	1 (5)	1 (5)	
Sample Day 3	Room	63 (197)	24 (133)	23 (130)	
	Bathroom	169 (319)	71 (227)	66 (225)	
	Care area	6 (15)	0 (0)	1 (5)	
	Patient area	15 (31)	0 (0)	3 (10)	

Abbreviations: CFU, colony-forming units; SD, standard deviation.

Table 3. ***Clostridioides difficile*** **Recovery Rate by Study Arm, Study Day, and Sample Location**

	Infected	Colonized	Control	*P*
Sample Day and Area	n (%)	n (%)	n (%)	Infected vs Control, Colonized vs Control, Infected vs Colonized
	N = 71	N = 82	N = 94	
Total				
Room	44 (62)	35 (43)	14 (15)	<.01, <.01, .08
Bathroom	40 (56)	34 (41)	12 (13)	
Care area	6 (8)	1 (1)	1 (1)	
Patient area	20 (28)	4 (5)	3 (3)	
Sample Day 1				
	N = 28	N = 32	N = 34	
Room	20 (71)	12 (38)	6 (18)	
Bathroom	16 (57)	12 (38)	6 (18)	
Care area	2 (7)	1 (3)	0 (0)	
Patient area	8 (29)	3 (9)	0 (0)	
Sample Day 2				
	N = 25	N = 30	N = 31	
Room	15 (60)	17 (57)	3 (10)	
Bathroom	16 (60)	16 (53)	2 (7)	
Care area	1 (4)	0 (0)	0 (0)	
Patient area	7 (28)	1 (3)	1 (3)	
Sample Day 3				
	N = 18	N = 20	N = 29	
Room	9 (50)	6 (30)	5 (17)	
Bathroom	9 (50)	6 (3)	4 (14)	
Care area	3 (17)	0 (0)	1 (3)	
Patient area	5 (28)	0 (0)	2 (7)	

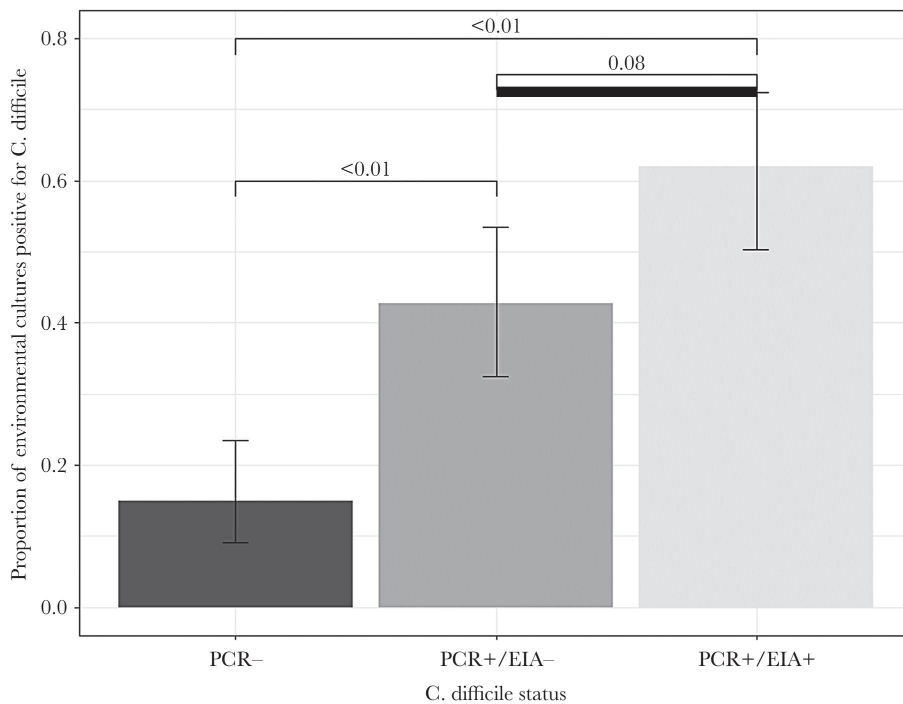

Figure 1. Comparison of proportion of positive patient rooms by Clostridium *difficile* status. Error bars represent 95% confidence intervals. EIA, enzyme immunoassay; PCR, polymerase chain reaction.

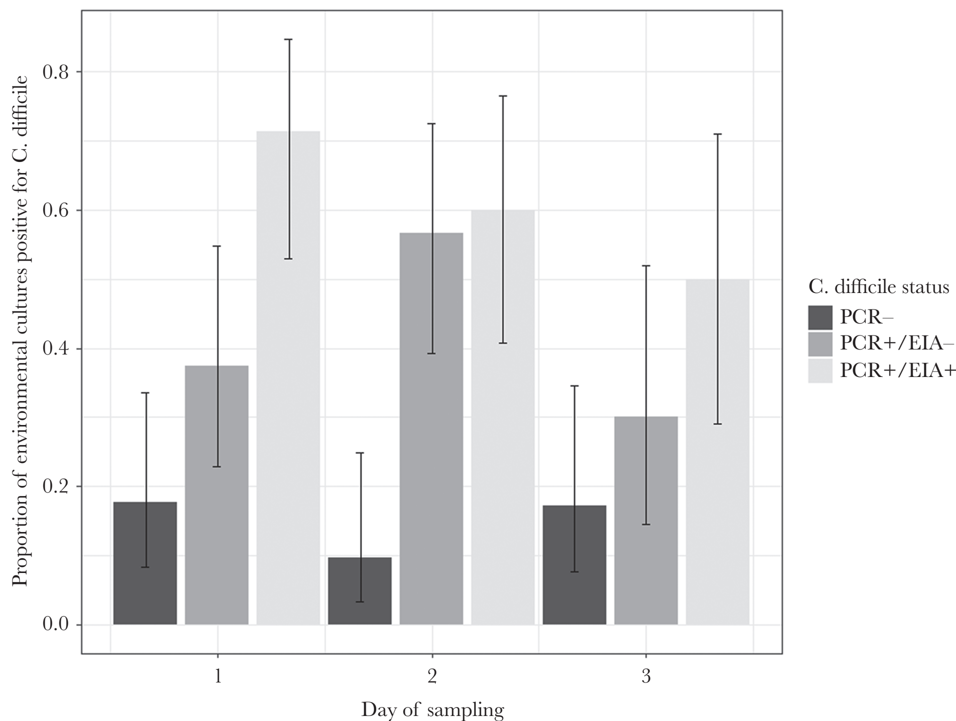

Figure 2. Proportion of positive patient rooms over time, by *Clostridium difficile* status. Error bars represent 95% confidence intervals. EIA, enzyme immunoassay; PCR, polymerase chain reaction.

patient rooms had more than 1 ribotype recovered from the environment.

DISCUSSION

Shedding of *C difficile* spores from infected individuals contaminates the hospital environment and contributes to infection transmission [4]. However, the relative contribution of colonized individuals on healthcare environmental contamination is poorly understood. Contact precautions are used to prevent the transient inoculation of healthcare provider hands and clothing from the patient and the healthcare environment. Current guidelines do not recommend the use of contact precautions for asymptomatic *C difficile* carriers nor offer recommendation due to the lack of data [6–8]. Our study is the first to prospectively compare *C difficile* environmental contamination between infected and colonized patients. We determined that environmental contamination by colonized individuals was common and significantly greater than control rooms. In contrast, we did not identify a significant difference between infected and colonized patient rooms. Our findings are bolstered by a high level of genotypic concordance between patient and environmental *C difficile* isolates via ribotyping in infected and colonized study arms.

Although colonized and infected patient room contamination was not different overall, they differed in contamination of the bathroom (40 of 66 positive samples for infected patient rooms, 34 of 39 for colonized, and 12 of 16 for control). Because the proportion of contaminated samples for colonized patient bathrooms was high and contamination in the patient and care areas was low, contact precautions or other infection prevention measures (eg, environmental disinfection) might be focused specifically for the bathroom area in colonized patient rooms. Our data also support using dedicated bathrooms for patients with known *C difficile* colonization. Of note, the majority of contaminated samples for all 3 groups were from the bathroom.

Another important finding was the high baseline of contamination in control patient rooms. Fifteen percent of control patient rooms and 12% of control patient bathrooms were contaminated. These findings were observed in the setting of close collaboration between infection prevention and EVS, routine monitoring, and feedback of surface disinfection adherence, use of bleach for all surface disinfection, and use of UV-C for terminal disinfection of high-risk rooms. Nevertheless, data from several published studies demonstrate that cleaning adherence is low (47%–76% surfaces were deemed clean) [13–15]. Overall, our findings further validate the ongoing concern that the healthcare environment harbors epidemiologically important pathogens and confirm that targeting specific, high-risk settings for enhanced disinfection will not eliminate this risk.

Our overall (43%–62% of PCR^+ rooms) and location-specific recovery rates (eg, 41%–56% in the bathroom) were higher than previous studies. Several previously published studies identified *C difficile* in approximately 15% of samples overall and 17%–27% of samples from the patient bathroom [12, 16, 17]. In contrast, Davies et al [18] and Gilboa et al [19] identified *C difficile* in

30%–70% of samples from *C difficile* patient rooms, and Curry et al [20] identified *C difficile* in 5 of 6 colonized patient rooms but not primarily in the bathroom [18, 19]. Although differences in recovery rate could be related to patient mix and timing of sampling, we suspect that the sampling technique contributed to these differences. Davies et al [18], Gilboa et al [19], and our study used the sponge and stomacher technique, which allows for sampling of a much larger surface area than contact plates or cotton swabs. In addition, Curry et al did not include the bathroom floor as a sample location as our study did, which likely contributed to our difference in findings in the bathroom.

Our study has several limitations. First, the PCR and EIA test results served as surrogates for *C difficile* infection, colonization, and controls; thus, some patients may have been improperly categorized. However, to address the potential for misclassification of infected patients as EIA-/asymptomatic *C difficile* carriers, chart reviews were completed on of PCR^+/EIA^- patients and determined the likelihood in our cohort was low. Of note, a minority of these patients met the criteria of ≥3 stools in 24 hours, a minority were treated for CDI, and more than 85% had an alternative cause of diarrhea. Second, diarrhea as an inclusion criterion limited our colonized cohort to patients with *C difficile* symptoms with an alternative cause. This diarrhea may have increased the shedding of colonized patients and therefore inflated our results. Third, routine disinfection was not monitored, although our institution has a singular disinfection protocol for patient rooms when housing patients under suspicion for *C difficile* infection. Fourth, sporicidal disinfectants were used in study rooms and surfaces, with the exception of the floors. Because our bathroom sample included flooring around the toilet, our numbers may be inflated for this sample area. Finally, patient acquisition and outcomes were not measured.

CONCLUSIONS

In conclusion, our findings demonstrated that patients colonized with *C difficile* contaminate the hospital environment at a frequency and amount that is almost equal to the level of contamination observed in rooms housing patients with CDI. Contrary to current guidelines, our findings suggest that contact precautions may be needed to prevent transmission of *C difficile* from the environment of colonized patient rooms. In addition, these findings suggest the use of sporicidal agents in all patient rooms, notably floors, should be studied because it may be a critical piece of environmental disinfection.

Acknowledgments

Financial support. This work was funded by the Centers for Disease Control and Prevention: Duke-UNC Prevention Epicenter Program for Prevention of Healthcare-Associated Infections (Grant Number 1U54-CK000483).

Potential conflicts of interest. All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

CONCLUSIONS

References

1. Miller BA, Chen LF, Sexton DJ, Anderson DJ. Comparison of the burdens of hospital-onset, healthcare facility-associated *Clostridium difficile* infection and of healthcare-associated infection due to methicillin-resistant *Staphylococcus aureus* in community hospitals. Infect Control Hosp Epidemiol **2011**; 32:387–90.
2. Lessa FC, Mu Y, Bamberg WM, et al. Burden of *Clostridium difficile* infection in the United States. N Engl J Med **2015**; 372:825–34.
3. Turner NA, Anderson DJ. Hospital infection control: *Clostridioides difficile*. Clin Colon Rectal Surg **2020**; 33:98–108.
4. Vonberg RP, Kuijper EJ, Wilcox MH, et al. Infection control measures to limit the spread of *Clostridium difficile*. Clin Microbiol Infect **2008**; 14:2–20.
5. Dubberke ER, Reske KA, Noble-Wang J, et al. Prevalence of *Clostridium difficile* environmental contamination and strain variability in multiple health care facilities. Am J Infect Control **2007**; 35:315–8.
6. McDonald LC, Gerding DN, Johnson S, et al. Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis **2018**; 66:e1–48.
7. Kelly CR, Fischer M, Allegretti JR, et al. ACG clinical guidelines: prevention, diagnosis, and treatment of *Clostridioides difficile* infections. Am J Gastroenterol **2021**; 116.
8. Dubberke ER, Butler AM, Reske KA, et al. Attributable outcomes of endemic *Clostridium difficile*-associated disease in nonsurgical patients. Emerg Infect Dis **2008**; 14:1031–8.
9. Polage CR, Gyorke CE, Kennedy MA, et al. Overdiagnosis of *Clostridium difficile* infection in the molecular test era. JAMA Int Med **2015**; 175:1792–801.
10. International Organization for Standardization I. Sterilization of medical devices — microbiological methods part 1. ISO Standard **1995**; 11737–1. https://www.iso.org/standard/38711.html.
11. Bidet P, Barbut F, Lalande V, et al. Development of a new PCR-ribotyping method for Clostridium difficile based on ribosomal RNA gene sequencing. FEMS Microbiol Lett **1999**; 175:261–6.
12. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol **1995**; 33:2233–9.
13. Weber DJ, Anderson D, Rutala WA. The role of the surface environment in healthcare-associated infections. Curr Opin Infect Dis **2013**; 26:338–44.
14. Boyce JM, Havill NL, Dumigan DG, et al. Monitoring the effectiveness of hospital cleaning practices by use of an adenosine triphosphate bioluminescence assay. Infect Control Hosp Epidemiol **2009**; 30:678–84.
15. Carling PC, Briggs JL, Perkins J, Highlander D. Improved cleaning of patient rooms using a new targeting method. Clin Infect Dis **2006**; 42:385–8.
16. Turner NA, Warren BG, Gergen-Teague MF, et al. Impact of oral metronidazole, vancomycin, and fidaxomicin on host shedding and environmental contamination with *Clostridioides difficile*. Clin Infect Dis **2021**. doi:10.1093/cid/ciab473.
17. Widmer AF, Frei R, Erb S, et al. Transmissibility of *Clostridium difficile* without contact isolation: results from a prospective observational study with 451 patients. Clin Infect Dis **2017**; 64:393–400.
18. Davies K, Mawer D, Walker AS, et al. An analysis of *Clostridium difficile* environmental contamination during and after treatment for *C difficile* infection. Open Forum Infect Dis **2020**; 7:11.
19. Gilboa M, Houri-Levi E, Cohen C, et al. Environmental shedding of toxigenic *Clostridioides difficile* by asymptomatic carriers: a prospective observational study. Clin Microbiol Infect **2020**; 26:1052–7.
20. Curry SR, Muto CA, Schlackman JL, et al. Use of multilocus variable number of tandem repeats analysis genotyping to determine the role of asymptomatic carriers in *Clostridium difficile* transmission. Clin Infect Dis **2013**; 57:1094–102.