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Major Article

Hospital-associated *Clostridium difficile* infection and reservoirs within the hospital environment

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Background: *Clostridium difficile* infection (CDI) is a leading cause of hospital-associated infections. Antibiotic stewardship, environmental disinfection, and reduction of transmission via health care workers are the major modes of CDI prevention within hospitals.

Methods: The aim of this study was to evaluate the role of the environment in the spread of CDI within hospital rooms. Bed tracing of positive-CDI inpatients was performed to detect the strength of association to specific rooms. Environmental cultures were conducted to identify adequacy of environmental *C difficile* (CD) spores. Whole-genome sequencing was performed to evaluate the degree of CD relatedness.

Results: Bed tracing performed for 211 CDI patients showed a limited list of high-burden rooms. Environmental cultures for surfaces disinfected with a sporicidal agent were almost entirely negative, whereas the floors were positive for CDI in 15% of the studied patient rooms. Whole-genome sequencing did not detect any close genetic relatedness.

Conclusions: Unlike in an outbreak setting, bed tracing did not yield conclusive results of room reservoirs. The C diff Banana Broth culture was inexpensive, sensitive, and easy to incubate under aerobic conditions. Sporicidal disinfectants were effective in eliminating CD from the environment. CD spores were found on floors and hard-to-clean surfaces.

Clostridium difficile infection (CDI) is a leading cause of hospital-associated (HA) infections. The overall number of reported CDI has shown to be on the rise, particularly HA-CDI cases.[1] HA-CDI is driven by using antibiotics, environmental contamination, and transmission by health care workers.[2] Individuals with CDI or colonization shed the spores by fecal contamination of the environment. Contamination of surfaces, devices, and the health care workers' body or attire could serve as a reservoir for *C difficile* (CD) spores.[3] These spores persist on surfaces for long periods and can be transmitted to patients typically via the fecal–oral route.[3] CD spores are resistant to various cleaning products and alcohol sanitizers, which results in widespread dissemination of the spores in closed settings such as health care facilities.[4,5] Asymptomatic CD colonization can also be seen in 2%-3% of healthy individuals and 10%-25% of hospitalized patients.[6] A susceptible host and an adequate exposure to CD spores are required for CDI to occur.[7]

Hospitalized patients are reported to have increased risk for HA-CDI if the prior occupant of the room was CD positive, their roommate had positive CDI, or the room was previously occupied by a person who was on antibiotics.[6,7] CD spores can persist in the environment for up to 5 months and can be isolated from beds, bed rails, walls, and floors of the hospital rooms that were previously occupied by a CDI patient.[6-8] Long survival time further facilitates dissemination of CD spores to surfaces beyond the immediate patient environment.[8] CD has shown to be cultured from 49% and 29% of the surfaces of hospital rooms that were previously occupied by a CD-positive patient and asymptomatic carriers, respectively.[6] Ninety percent of the floors of bathrooms and the corners of the isolation rooms in the hospital can be contaminated by CD and is 1 of the most frequently recovered pathogens from the hospital floor.[8,9] It is possible for some frequently

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touched objects, such as a call button, linens, and medical devices, that come in contact with the floor promote the transmission of pathogens to the patients' hands.[9] A study demonstrated that there was a minimum of 1 pathogenic organism in 39% of the patients' hands and ≥2 pathogens in 8% of the patient's hands, of which 14% were CD positive.[10]

Cleaning hospital rooms with a 1:10 dilution of bleach or sporicidal agents has been proven to be effective in reducing the environmental burden of CD.[6,8] CDI spread can be reduced if room cleaning is associated with handwashing, contact precautions, and isolation of patients on presentation of symptoms.[6,8] These precautions are recommended only for rooms and or CDI-positive patients.[8] It is important to note that patients can remain asymptomatic and contribute to contaminating the hospital environment.[8] To further understand the spread of CDI within the hospital, we investigated the role of the hospital environment in the spread of CDI by performing patient room tracing and environmental culturing.

METHODS

This study was conducted in a 495-bed single academic hospital, which is part of a large university-affiliated medical system. The aim of the study was to investigate the role of the hospital environment as a potential reservoir for transmission of CDI. Using electronic medical records (EMRs), we identified all patients with CD-positive (polymerase chain reaction) testing (either hospital or community acquired [CA]) between January 1, 2016 and December 31, 2016. We conducted bed tracing to identify the movement of patients within the hospital to identify rooms that had a high occupancy of positive patients. Environmental culturing was performed using C diff Banana Broth media (Hardy Diagnostics, Santa Maria, CA. http://hardydiagnostics.com/cdiff/) to detect reservoirs of CD spores and vegetative cells. The study was approved by the University institutional review board as a quality improvement study.

Data collection

The details on CDI-positive patient age, sex, race, death date (if applicable), HA-CDI or CA-CDI, Charlson's comorbidity index,[11] readmission and frequency of readmission, use of antibiotics and proton pump inhibitors (PPIs) during hospital admission, CDI-positive test date, history of CDI in 2014 or 2015, length of hospital stay, and patient movement within the hospital were collected manually from the EMR. Cerner PowerChart software (Cerner Corp., Kansas City, MO) was used for medical record abstractions and the TheraDoc (TheraDoc, Charlotte, NC) system was used for positive patient identification and bed tracing. A gap of <48 hours was considered as the same admission, and a patient was considered as readmitted if he or she was admitted 90 days prior to his or her first 2016 CD-positive test stay.

Data analysis

Statistical analysis was performed using SAS software version 9.1 (SAS Institute, Cary, NC). The Fisher exact test was used to compare HA-CDI and CA-CDI (outcome variables) with different parameters, such as history of CDI, readmission, use of antibiotics, and PPIs (exposure variables). All of the variables analyzed were categorical variables with 2 levels. The Kruskal-Wallis test was used to identify the differences in continuous variables, such as age, confidence interval, and length of stay (LOS) between the HA-CDI and CA-CDI patients. The results were statistically significant if the P value was ≤0.05. A total of 196 (111 HA-CDI and 85 CA-CDI) of the 211 CD-positive inpatients were included in the final analysis of baseline characteristics, as 15 were not classified as HA-CDI or CA-CDI on EMRs, but were manually classified, depending on the time of admission and date of the CD-positive test. A positive laboratory result after 48 hours of hospital admission or hospital admission in the previous 90 days were classified as HA-CDI.

Bed tracing procedure

In this study, bed tracing was used as a procedure to track the movement of patients within the hospital. Patient rooms with >9 CDI patients were identified from the data collected within the hospital and were classified as high-density rooms. A heat map was created, using R programming language to graphically represent the density of CDI patients, by plotting a graph of high-density rooms against the number of weeks in 2016.

Environmental culturing procedure

Environmental culturing was performed in specified areas of the hospital. This included 53 intensive care unit rooms and 28 rehabilitation rooms. Floor cultures were completed in the 28 rehabilitation rooms. In addition, 20 clean, stored wheelchairs in the rehabilitation area were also cultured. Contact and droplet precautions were followed while culturing the rooms and were noted, if mentioned, for any of the patient rooms. Empty rooms, with or without beds, were also noted while culturing. EMRs were checked to determine if any patient tested positive for CD at the time of culturing.

C diff Banana Broth (10 mL of Brucella broth with thioglycolic acid and L-cystine) is a sensitive aerobic CD environmental culture.[12] Sterile cotton swabs were moistened with the broth prior to environmental culture. After obtaining the cultures, the swabs were inserted into the culture tubes and were tightly closed. The broth tubes were then incubated at 34°C for 7 days and were checked for color change (turned yellow) every 24 hours. Confirmatory testing was performed to verify that these were truly CD spores. A positive broth tube was streaked on cycloserine, cefoxitin, fructose, and egg yolk agar plates and incubated under anaerobic conditions at 35°C for 72 hours, after which Gram stain was used to confirm the morphology. All isolates with suspected CD were tested using an anaerobic biochemical identification kit (Remel RapID ANA II System, Thermo Fisher Scientific, Waltham, MA. https://www.thermofisher.com/order/catalog/product/R8311002) for anaerobic identification. The wells were filled with the liquid culture broth and incubated per the user manual. The reading of biochemical reactions is based on the manufacturer guide.

The culture sites for the intensive care unit rooms included high-touch surfaces, such as bed rails, table, monitors, pumps, vents, tube feeding and infusion pumps, oxygen fixtures, cable insertion to the monitor, and call buttons. One broth tube was used per room to swab all of the preceding sites. Rehabilitation rooms consisted mostly of private single-bed rooms with attached toilets. However, a few of the rooms were semiprivate, with 2 beds, separated from each other by a curtain. Patients in the semiprivate rooms had a shared toilet. For culturing the rehabilitation rooms, 2 broth tubes were used per private or semiprivate room, which included 1 for the floor, encompassing corners of the room and toilet room floor, and another for the surfaces, which included toilet seats, phones, call buttons, bed rails, and tables. The culture sites for the wheelchair included the most frequently touched areas by the patient while using the wheelchair. One Banana Broth tube was used per wheelchair.

Molecular typing procedure

Whole-genome sequencing (WGS) was performed on all positive environmental CD isolates at the Microbial Genomics Epidemiology Laboratory, University of Pittsburgh, Pennsylvania. A phylogenetic tree based on single-nucleotide polymorphisms (SNPs) was generated using CD sequence type 1 (ST1) as a reference strain. The

Table 1
Analysis of baseline characteristics of HA-CDI versus CA-CDI

Characteristics	HA-CDI (N* = 111)	CA-CDI (N* = 85)	*P* value
LOS	19.7 d	6.6 d	<.0001
Charlson's CI	4.1	4.6	.35
Age	63	66	.25
History of CDI	9.90%	21.20%	.04
PPI use	76.6%	61.2%	.03
Antibiotic use	99.1%	96.5%	.3
Previous admission within 90 d	9.9%	21.2%	.04
Sex			
Male	60.4%	48.2%	.1
Female	39.6%	51.8%	—
Race			
White	85.6%	76.5%	.1
All-cause mortality	28.8%	21.2%	.1

CA-CDI, community-acquired *Clostridium difficile* infection; *CDI*, *C difficile* infection; *CI*, comorbidity index; *HA-CDI*, hospital-associated *C difficile* infection; *LOS*, length of stay; *PPI*, proton pump inhibitor.
*N = the number of patients considered for the analysis.

sequence type (ST) was determined by querying the pubMLST database (http://pubmlst.org/cdifficile/). The strains with <7 SNPs between the pairs were considered to be highly related genetically.

RESULTS

An average of 1,200-1,500 tests for CD are conducted annually at our institution, in which a steady increase in the rate of positive tests annually, from 6%-15%, has been documented. In 2016, 211 patients tested positive for CDI. Of that number, 115 patients (55%) were classified as HA-CDI and 96 patients (45%) were classified as CA-CDI. The main characteristics of patients with CDI are summarized in **Table 1**. There was no statistically significant difference between HA-CDI and CA-CDI in regard to age and comorbidity score. However, 60% of our HA-CDI patients were men who had an average LOS of 25 days, as compared to our CA-CDI patients, in which 48% were men with an average LOS of 9 days. Patients with CA-CDI (22.2%) were more likely to have a previous history of CDI, as compared to HA-CDI patients (9%) ($P = .04$). A significant majority of patients with HA-CDI or CA-CDI received PPIs (76.6% and 61.2%, respectively) and antibiotics (99.1% and 96.5%, respectively). There were statistically more readmissions in CA-CDI patients (19% or 21.2%) versus HA-CDI patients (13% or 9.9%) ($P = .04$).

Bed tracing results

All hospital rooms were reviewed. However, we limited the analysis to inpatient rooms and excluded emergency rooms, radiology, operation, or postoperative rooms. The most frequently used rooms for CD patients were within medical units (470 patients in 220 beds) and critical care units (238 patients in 53 beds). There were fewer patients in rehabilitation or surgical units. There were 13 rooms that had ≥9 CDI patients. These rooms were not in close proximity within the hospital except for 2 rooms within critical care. **Figure 1** shows a heat map summarizing the results of bed tracing.

Environmental culturing results

All 53 intensive care rooms cultured were negative**.** However, 12 rehabilitation unit cultures were positive (2 from the room surfaces and 10 from floor cultures). Two previously cleaned wheelchairs that were in the rehabilitation storage area have shown positive results. **Figure 2** illustrates the morphology of the growth of CD and the Banana Broth cultures done for this study. A positive Banana Broth environmental culture was indicated by change in color from red to yellow, as seen in **Figure 2A.** The CD colonies growing on agar plates and the gram-positive rods on Gram stain are shown in **Figure 2B** and **C.** A specific biochemical reaction noted for CD is shown in **Figure 2D**.

Molecular typing results

Figure 3 shows a phylogenetic tree of all of the 14 positive CD strains**,** and **Table 2** shows the pairwise comparison of the genomes belonging to the same STs. The 14 CD isolates belong to 8 STs, out of which 3 of the STs are highly genetically related, CD08130 and CD08132 (**Fig 3, box i**) have 7 SNPs between the pair and are considered highly genetically related; CD08127, CD08129, and CD08128 (**Fig 3, box ii**) have 0-1 SNPs between pairs; and CD08126 and CD08138 (**Fig 3, box iii**) have 1 SNP between the pairs and are considered to be highly genetically related. Two STs, 15 and 2, have 499 and 511 SNPs, respectively, between the pair and are genetically unrelated. CD08135 belongs to a ST that has not been defined in the pubMLST database. CD08134, CD08135, and CD08125 are genetically unrelated to any isolate with >10,000 SNPs between isolate pairs. Isolates within each of ST1, ST42, and ST103 are genetically related but are genetically unrelated to other isolates with different STs (>10,000 SNPs).

DISCUSSION

Prevention of HA-CDI is a major task for health care systems by applying better infection prevention strategies and antimicrobial stewardship. Our study was focused on evaluating the role of the environment in the spread of CDI by performing bed tracing and environmental cultures. Bed tracing revealed high-density CD patient rooms, but it could not answer if this was related to CD spread or nursing-preferred placement of CDI patients in specific rooms to facilitate their care. This could challenge some of the previous studies that suggested increased risk of developing CDI if the room was occupied by a CD patient or even patients who received antibiotics.[7,13] As total random placement of patients is not practically possible, environmental culture that match subsequent patient isolate may be a better estimate of transmission owing to environmental contamination.

Our patients are consistent with typical CD patients with older age (mean age of >60 years), who have a high CI score (mean >4), and who have extensive PPI and antibiotics use.[14,15] Readmission within 90 days prior to CDI diagnosis was significantly more common in CA-CDI patients. However, the causes for the spread of CDI within the hospital environment are still not clearly understood. The WGS data showed potential different sources of CD spores not directly related to CD cases.[16] However, there is a strong relationship between admission to the health care facility and subsequent diagnosis of CDI.[15,17,18] The current National Health Safety Network definition does not differentiate between colonization and symptomatic infection.[19] It is clear that a major part of CDI is a continuum between community and health care rather than 2 distinct categories.[20]

In our study, approximately 50% of the room floors were positive for CD on confirmatory tests. These results are consistent with other studies that floors are common reservoirs for CD.[8,9] We cultured the room corners where we believed the air current would push particles and spores. Our cultures from highly touched surfaces were almost 100% negative. This is consistent with improved environmental disinfection and daily cleaning reported in other studies.[21] In our institution, floors are not routinely disinfected by a sporicidal agent (quaternary ammonium) as opposed to the surfaces that are disinfected with a sporicidal agent (chlorine-based tablets, 48% sodium dichloro-s-triazinetrione). Contamination of the patient and his or her immediate environment could occur by the patient walking on the floor without shoes or objects falling on the floor (most

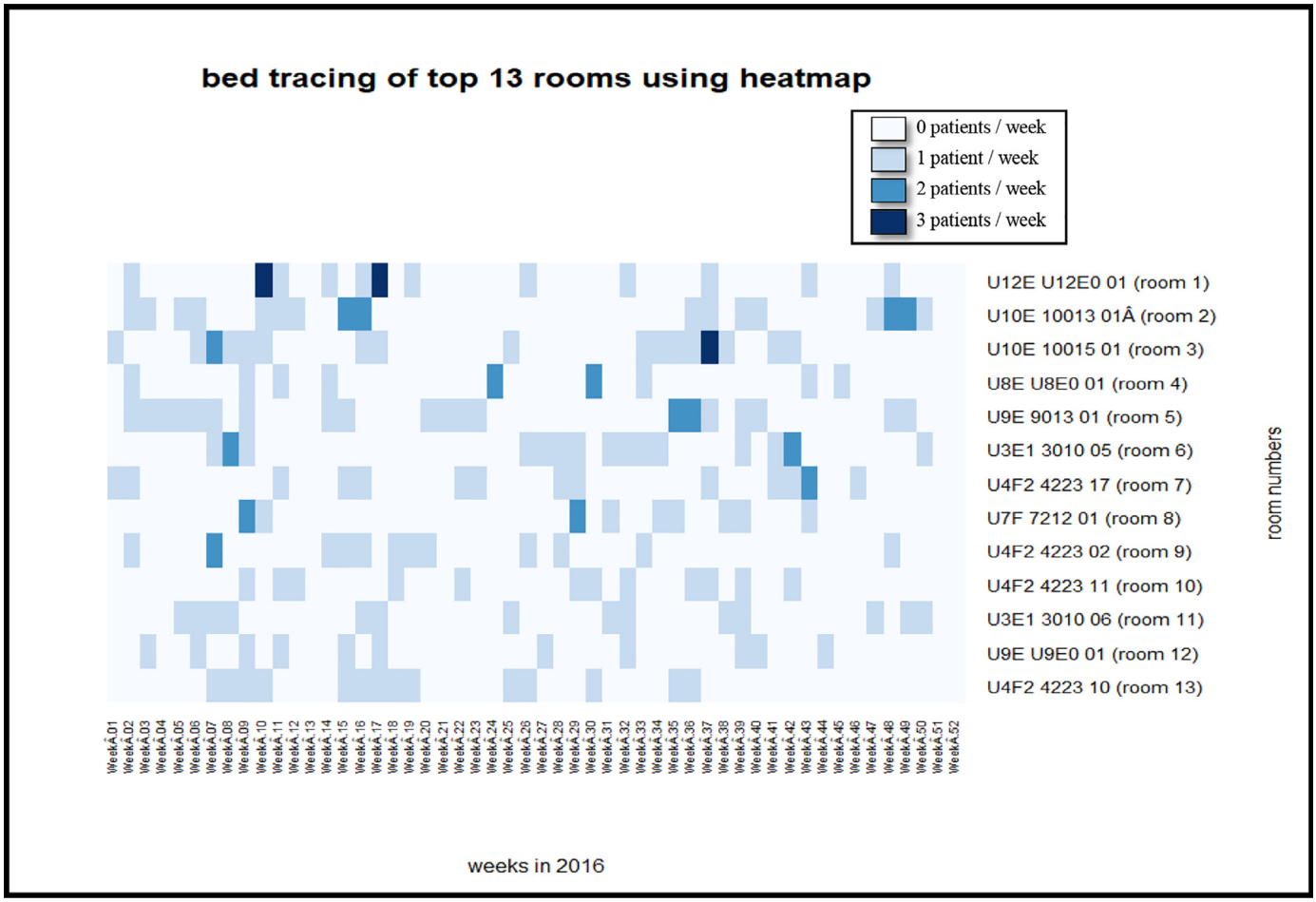

Fig 1. A heat map shows wide spread of CD-positive rooms within the hospital with little specific focus. The medical-surgical rooms are 1-5, 8, and 12, and the rest are intensive care rooms. *CD*, *Clostridium difficile*.

important, pillows, bed sheets, and other devices). Although it is hard to keep floors clean, it is important to consider sporicidal disinfectant with any increased CDI incidence or suspected hospital transmission.

WGS of the isolates showed no significant relationship between the samples, although 8 out of 14 samples were genetically related. This result was consistent with another study on WGS of isolates from patients in a hospital setting. It showed only 35% of the CDI cases to be genetically related to at least 1 previous case.[16] Hence, we can assume that apart from symptomatic and asymptomatic patients, environmental sources, such as food, water, and animals, can act as a reservoir and contribute to the spread of the infection.[22] As the genome of CD is large and diverse, the interpretation of clonality should be using both phylogenetic and clinical findings.[2]

Health care professionals can also play an important role in the transmission of CD spores. Hand hygiene ([HH] washing of hands with soap and water) protocol should be followed by both hospital staff and patients. Health care professionals should actively take part in educating patients and visitors. Patients should be advised to avoid

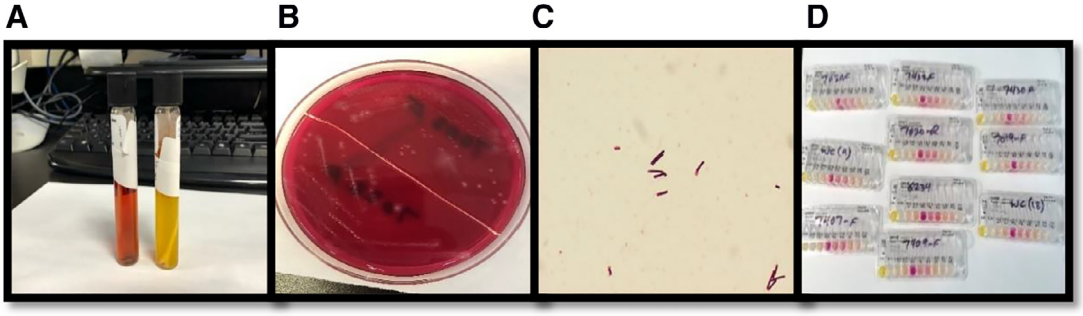

Fig 2. (A) Light-colored (yellow) positive Banana Broth on the right and dark-colored negative control on the left. (B) Typical growth of CD on CCFA agar plates after 24-hour anaerobic incubation. (C) Direct Gram's staining from CCFA agar showing typical gram-positive rods of CD. (D) Remel RapID ANA II system cartridge for anaerobic biochemical identification showing positive results typical for CD. *CCFA*, cycloserine, cefoxitin, fructose, and egg yolk; *CD*, *Clostridium difficile*.

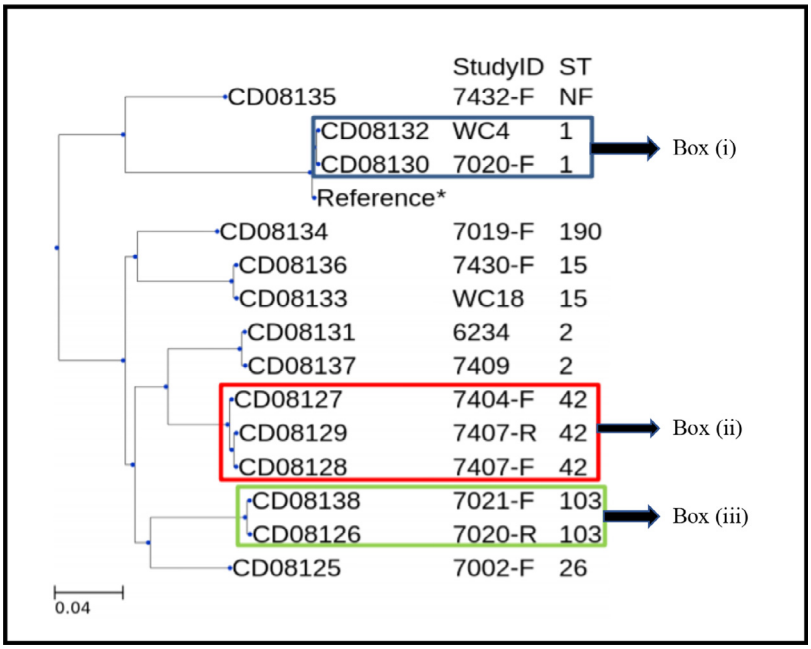

Fig 3. Boxes (i, ii, and iii) represent the highly genetically related CD isolated with <7 SNPs. CD, *Clostridium difficile*; *SNP*, single-nucleotide polymorphism.

Table 2
WGS of positive environmental cultures

Isolate 1	Isolate 2	ST	SNPs
CD08132	CD08130	1	7
CD08128	CD08127	42	0
CD08129	CD08127	42	1
CD08129	CD08128	42	1
CD08138	CD08126	103	1
CD08136	CD08133	15	449
CD08137	CD08131	2	511

NOTE. Pairwise comparison of genomes belonging to the same sequence types with significant variability.
CD, *Clostridium difficile*; *SNP*, single-nucleotide polymorphism; *ST*, sequence type; *WGS*, whole-genome sequencing.

placing high-touch objects on the floor. One study showed that CDI can be reduced by educating patients about HH.[10]

This study has certain limitations. First, HH compliance was not measured by the researchers. However, our internal audits showed HH compliance within rehabilitation units at the same time frame was 85% (910 compliant observations out of 1,071). Second, nonrandom patient placement may have affected bed tracing results. Third, the number of cultures performed is small (130 cultures), and this was a single center experience for which the results may not reproducible.

CONCLUSIONS

Environmental culturing coupled with bed tracing are simple and effective tools to evaluate CD spread within the hospital environment. Banana Broth is an inexpensive, easy-to-perform environmental culture method that could be performed in aerobic condition, which could be of great help for infection prevention efforts. The floors of the patient rooms may be reservoirs for CD that could result in the spread of CDI within hospitals. A sporicidal floor disinfectant should be used either routinely or to be implemented in any increased CDI incidence.

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