Successful Removal of *Clostridioides Difficile* Spores and Pathogenic Bacteria From a Launderable Barrier Using a Commercial Laundry Process

Infectious Diseases: Research and Treatment
Volume 13: 1–6

DOI: 10.1177/1178633720923657

Edmond Anderson Hooker[1], David Ulrich[2] and Dane Brooks[3]

[1]Department of Health Services Administration, Xavier University, Cincinnati, OH, USA. [2]MedRest, Inc, Sunman, IN, USA. [3]Research and Development Department, Q Laboratories, Cincinnati, OH, USA

ABSTRACT:

BACKGROUND: To properly clean and disinfect hospital mattresses, bed manufacturers recommend a 3- to 6-step process to remove all pathogenic bacteria. An alternative is to use a removable barrier on the mattress, which is laundered after each use. The current study was to determine efficacy of a commercial laundry process in eliminating *Clostridioides difficile (C diff)* spores, *Mycobacterium terrae (M terrae)*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa (P aeruginosa), Klebsiella pneumoniae (K pneumoniae)*, and *Escherichia coli (E coli)* from a barrier.

METHODS: A test barrier received 3 unique microbial suspensions in separate locations, each suspension having a known quantity of specific microorganisms: *C diff* spores, *M terrae*, and a mixed suspension of MRSA, *S aureus, P aeruginosa, K pneumoniae*, and *E coli*. A wash load contained the test barrier and 11 additional ballast barriers. Various soils were spread onto the barriers to simulate heavy soiling that may occur in a wash load: Each barrier received a small amount of mixed soil, 50% received urine, 25% received blood, and 25% received a large amount of additional mixed soil. The load was laundered using 71°C (160°F) water, detergent, and chlorine bleach, with final drying at 71°C (160°F). After laundering, remaining colony-forming units (CFUs) of each microorganism were counted at the applied locations. Each test was replicated 3 times. Industry-accepted methods were used to produce suspensions, apply inoculum, and recover organisms after laundering.

RESULTS: Before laundering, test barriers contained at least 7.0 $\log_{10}$ cfu/mL of each microorganism distributed over 103 cm^2. After laundering, in all cases, no residual CFUs were detected over the test area, resulting in greater than 6.0 $\log_{10}$ reductions for every organism. ($P < .05$).

CONCLUSIONS: Under extreme test conditions including the presence of soil, the laundry process removed all detectable pathogenic bacteria and spores from the barrier.

KEYWORDS: Laundry, bed barrier, ***C difficile***, spore, microorganisms

RECEIVED: November 6, 2019. **ACCEPTED:** April 11, 2020.

TYPE: Original Research

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was funded by Trinity Guardion, the manufacturer of the launderable barrier.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: E.A.H. serves as the medical director for Trinity Guardion, the manufacturer of the launderable barrier. D.U. is a consultant for Trinity Guardion.

CORRESPONDING AUTHOR: Edmond Anderson Hooker, Department of Health Services Administration, Xavier University, 3800 Victory Parkway, ML 5141, Cincinnati, OH 45207-5141, USA. Email: ehooker@mac.com

Introduction

Health care–acquired infections (HAIs) continue to be a problem in the United States and worldwide. The most recent data indicate some improvements in rates of HAIs; however, in the United States, there still were 687 000 HAIs in 2015, which resulted in 72 000 deaths.[1] While there continues to be a lot of efforts to improve hand hygiene, there is inadequate attention being given to disinfection of the hospital environment.[2] Numerous studies have linked the hospital mattress to HAIs including OXA-48-producing *Klebsiella pneumoniae* and CTX-M-15-producing *Enterobacter cloacae*.[3-5]

Most mattress covers today are made of polyurethane coated fabric and are typically cleaned and disinfected by manually wiping the surface 1 time with a health care disinfectant. Currently used disinfectants include quaternary ammonia compounds, sodium hypochlorite (bleach), hydrogen peroxide, and peroxyacetic acid (peracetic acid), hydrogen peroxide/peroxyacetic acid combination, and phenolics. While the disinfectants kill bacteria in the laboratory on hard surfaces, their performance in actual use on soft, hygroscopic mattress covers is limited by variability in the manual process, insufficient soil removal, and difficulty maintaining specified disinfectant dwell time. The use of quaternary ammonia achieved only a 1 $\log_{10}$ cfu reduction in bacteria.[6,7] Peroxyacetic acid use was associated with a 2 $\log_{10}$ cfu reduction in pathogenic bacteria, but only a 1 $\log_{10}$ cfu reduction in *Clostridioides difficile (C diff)*.[8,9] Sodium hypochlorite use also failed to eliminate *C diff* from hospital surfaces (1 $\log_{10}$ cfu reduction).[10,11]

Only a small percentage of hospitals preclean the mattress and rinse off the disinfectants, as recommended by many mattress manufacturers.[12] The failure to rinse these disinfectants off the mattress may be the reason for increasing rates of mattress damage. One study of acute care hospitals in Canada showed 32.5% of mattresses were damaged, and another in the United States found 26% were damaged.[13,14] In 2017, the United States Food and Drug Administration

(FDA), after receiving more than 700 reports of mattress failure, issued a safety notice advising hospitals to routinely inspect mattresses for damage.[15] ECRI Institute described mattress contamination and damage as 1 of their top health hazards for 2018 and 2019.[16]

A polyurethane coated fabric with bonded seams may be used as a barrier to protect the mattress from disinfectant-related damage. Laundering the barrier can eliminate issues with insufficient removal of pathogens from the patient surface and residual disinfectant on the mattress cover. A barrier provided by Trinity Guardion has been shown to decrease *C diff* infections (CDIs) in long-term acute care hospitals in the United States.[17] The goal of the current study was to determine the efficacy of a commercial laundry process in reducing bacterial and spore contamination on the barrier.

Materials and Methods

Microorganism and growth conditions

Three suspensions of microorganisms were produced. The first one contained methicillin-resistant *Staphylococcus aureus* (ATCC 33592), *Pseudomonas aeruginosa* (ATCC 15442), *K pneumoniae* (ATCC 10031), and *Escherichia coli* (ATCC 11229). The organisms were propagated on Tryptic Soy Agar with 5% Sheep Blood, incubated, isolated in Tryptic Soy Broth (TSB), incubated, centrifuged, and the pellet resuspended in 10-mL TSB. Initial organism concentrations were determined by performing 10-fold serial dilutions of each organism. Dilutions were plated in triplicate and incubated at 35°C for 48 hours. After incubation, colonies were enumerated and recorded as colony-forming unit (CFU)/plate with triplicate plates. The mean number was then multiplied by the dilution factor to determine CFU/mL of suspension.

The second suspension contained *Mycobacterium terrae* (ATCC 15755), which was propagated on M7H10 agar plates, incubated until sufficient growth was observed, harvested with phosphate-buffered solution (PBS), centrifuged, and the pellet resuspended in 5-mL TSB. Initial organism concentrations were determined by performing 10-fold serial dilutions in PBS. Dilutions were plated in triplicate and incubated at 35°C for 2 weeks. After incubation, colonies were enumerated and recorded as CFU/plate with triplicate plates. The mean was then multiplied by the dilution factor to determine CFU/mL of suspension.

The third suspension contained *C diff* spores (ATCC 43598 Strain 1470 Serogroup F). These were produced using the recommended procedures of the Environmental Protection Agency.[18]

Frozen stock was struck to CDC Anaerobic 5% Sheep Blood Agar (CABA) media plates, incubated anaerobically for 48 hours at 36°C, isolated in Reinforced Clostridial Medium (RCM), incubated anaerobically at 36°C for 24 hours, spread plated on CABA plates, verified for confluent lawn growth, and incubated anaerobically at 36°C for 10 days. Spore formation was verified and spore-to-vegetative cell ratio was estimated at more than 90% spores. Spores were harvested with PBS-T. After 3 wash cycles of vortex, centrifuge, and resuspension, the suspension was heat treated at 65°C for 10 minutes and evaluated for the spore-to-cell ratio, estimated at 90% or higher. Spore count of at least 10^8 was verified after plating 10-fold serial dilutions in PBS-T and incubating. Spore suspension was purified using a density gradient medium, followed by 3 wash cycles of vortex, centrifuge, and resuspension with PBS-T. The final spore suspension was evaluated for the spore-to-cell ratio, estimated to be at least 95%. Viable spore count of at least 10^7 was verified after plating 10-fold serial dilutions in PBS-T and incubating at 36°C for 24 hours. Based on the enumeration, the final suspension was diluted with PBS-T to achieve a desired concentration of 5.0×10^8 spores/mL. Using 10-fold serial dilutions, the final spore concentration was verified to be at least 2.0×10^8 spores/mL.

Soil urine and blood load on barrier

Representative bodily fluids and soils of the health care patient were included to provide additional challenge to the wash load. The soil load was a combination of Hucker's and Miles soils intended to simulate fecal material and provide the protein and hemoglobin elements of blood.[19,20] To maintain proper consistency, the distilled water in Hucker's was replaced by the Miles' rabbit blood/saline mixture and bovine serum. The ink was eliminated from Hucker's because color and staining properties were not relevant. The soil contained 10 g each of peanut butter, butter, flour, lard, dehydrated egg yolk, plus evaporated milk (15 mL), saline (3 mL), bovine serum (12 mL), dried milk powder (7.2 g), and rabbit blood mixed 1:1 with 0.85% saline (12 mL).

A wash load included 1 test barrier and 11 ballast barriers. Figure 1 shows the distribution of soil, urine, and blood for each wash load. The test barrier and all ballast barriers each received 3.0 g of soil. Three ballast barriers each received an additional 33.3 g. The test barrier and 5 ballast barriers received 100.0 mL of urine each. The test barrier and 2 ballast barriers each received 30.0 mL of rabbit's blood. All were allowed to dry for at least 24 hours.

Inoculation of test barrier. Organisms were applied to test barrier seams, which are the most difficult-to-clean areas. Prior to application, the *C diff* spore suspension was mixed with a bovine serum organic soil prepared according to EPA MLB SOP MB-31, combining 5% bovine serum albumin (BSA) stock, 7% yeast extract, 20% mucin stock, and 68% PBS spore suspension.[21] A 1.0-mL aliquot of each suspension was dispensed over a separate 4"× 4" inoculation area (Figure 1). The test microorganisms were spread uniformly over the area using a sterile spreader. The inoculum was allowed to air dry for at least 2 hours and was visibly dry before testing.

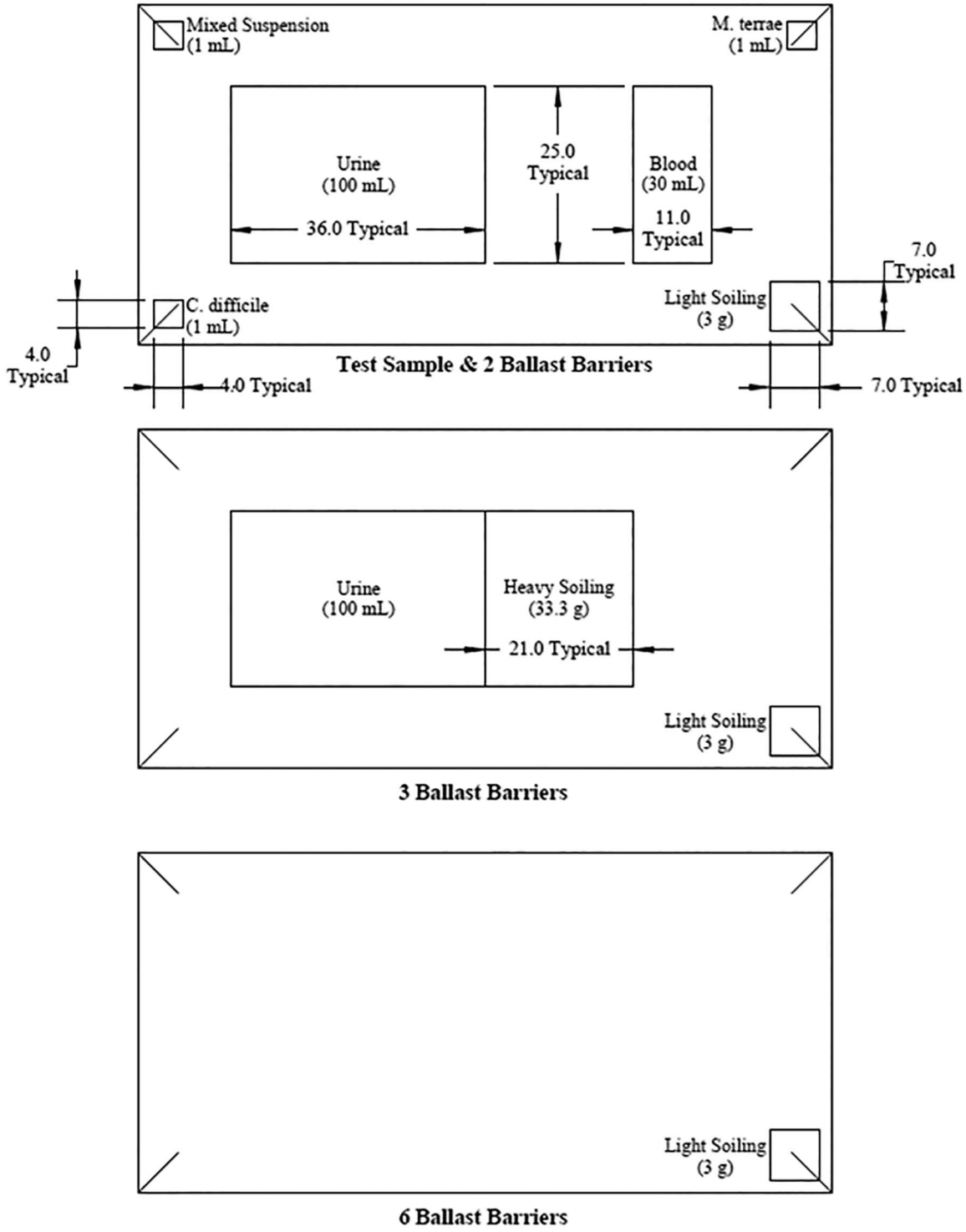

Figure 1. Application of spores, bacteria, soil, and urine on bed barriers.

Testing. Each test barrier was laundered and evaluated separately. There were 3 test barriers, 1 positive control, and 1 negative control that were in new condition, and 3 test barriers, 1 positive control, and 1 negative control that had undergone 200 laundry cycles (to represent end-of-life). Positive controls were inoculated in the same manner as test barriers, but not laundered. Negative controls were not inoculated and laundered.

For each test, 1 test barrier and 11 ballast barriers were laundered in a washer/extractor using laundry chemicals and water at the temperatures listed in Table 1. All barriers were dried at 71°C (160°F).

Residual organism determination. After laundering, the 4”× 4” inoculated area was wiped 3 times up and down and 3 times left and right with moderate hand pressure using a sterile

Table 1. Laundry process using a Milnor 30015M6J; 35-lb capacity.

STEP	OPERATION	DURATION (MINUTES)	MIN WATER TEMP (°F)[I]	WATER LEVEL	CHEMICAL	MIN CHEMICAL VOLUME (FL OZ/100 Lb)
1	Flush, 2 way	2	140	High	Detergent[a]	1
2	Break/detergent, 2 way	8	160	Low	Alkali[b]	4
					Detergent[a]	2
3	Bleach, 2 way	8	160	Low	12.5% chlorine bleach[c]	6 [II]
4	Rinse, 2 way	2	140	High	–	–
5	Rinse, 2 way	2	120	High	–	–
6	Rinse, 2 way	2	100	High	–	–
7	Sour, 2 way	4	90	Low	Souring agent[d]	1
8	Low Spd extract	6	Modify end of cycle for optimum water removal [III]			
	Total time	34				

[I]Specified temperature must be achieved and maintained for at least 2 minutes.
[II]Specified volume for bleach is the recommended starting point. Adjust to achieve a chlorine concentration between 125 and 150 ppm for the typical wash load.
[III]Repositioning the wash load numerous times with the drain open is most effective at eliminating trapped water and reducing drying time.
[a]Pulse Advance—Gurtler Industries, Inc.; South Holland, IL 60473 US.
[b]Pulse Ultra—Gurtler Industries, Inc.; South Holland, IL 60473 US.
[c]Sodium Hypochlorite—Gurtler Industries, Inc.; South Holland, IL 60473 US.
[d]NDT Sour—Gurtler Industries, Inc.; South Holland, IL 60473 US.

gauze pad wetted with 10 mL of sodium thiosulfate to neutralize any residual disinfectant and remove viable microorganisms. The gauze pad was placed in a sterile Stomacher bag with 90 mL of neutralizer. The bag was homogenized by stomaching and serial dilutions were prepared. The dilutions were plated and incubated as previously described. After incubation, colonies were enumerated and recorded as CFU/plate. The count was totaled for each dilution to calculate the total microorganisms recovered. Triplicate plates were multiplied by the dilution factor, and the mean was then reported as the remaining CFU/test barrier. This entire process was repeated for each microorganism type or group and each of the test barriers and controls.

Study controls. Positive controls were inoculated in the same manner as test barriers, but not laundered. These controls were used to verify organism recovery efficiency and to establish the total viable CFUs on the test barriers before laundering. The recovery target of at least 10^7 CFUs for each test organism was met.

Negative controls were not inoculated, but laundered to determine if any relevant CFUs were picked up in the laundering process. Negative controls were evaluated after all 3 test barriers were laundered and again after organism recovery. The recovery target of less than 100 CFU/mL for each test organism was met.

Wash water was sampled at the drain by aerobic plate count after completion of the bleach portion of the wash cycle (step 3). The acceptance criteria of less than 10 CFU/100 mL were met.

Total suspension viability. To verify the viability of the inoculum over the testing phase, the inoculum was enumerated at the start and end of the testing phase. Inoculum populations were determined by preparing serial dilutions of the challenge organism suspension in triplicate, plating, and incubating. Colonies were enumerated and recorded as CFU/plate to determine surviving organisms. The mean count for the triplicate plates was then multiplied by the dilution factor to calculate the microbial population (CFU/mL) of the control suspension. The target of less than 0.5 log variance from start to finish was met.

Neutralizer evaluation. Testing ensured that no components of the neutralizing procedure exerted an inhibitory effect on the test microorganisms according to ASTM International standards.[22] Testing included a neutralizer effectiveness test, neutralizer toxicity test, and organism viability test, and all met the target of less than 0.2 $\log_{10}$ difference between initial and final plate counts.

Organism recovery efficiency. Efficiency of organism recovery was validated by sampling the positive control 4 times. Recovery efficiency was above 93% in all cases except for 85.7% recovery on the end-of-life test sample for the mixed organism suspension.

Statistics

All statistics were performed using SPSS version 24 software (IBM, Armonk, NY). The significance was set at 0.05 for all tests. Counts of the microorganisms before and after washing were compared using the Mann–Whitney *U* test. Each investigation was repeated in triplicate both before inoculation and after washing.

Table 2. Summary of test results.

CHALLENGE ORGANISM	BED BARRIER PRELAUNDERING (NUMBER OF LAUNDRY CYCLES)	INOCULUM LOG_{10} CFU/ML (±SD)	BEFORE LAUNDERING POSITIVE CONTROL LOG_{10} CFU/ML	AFTER LAUNDERING ALL 3 REPLICATES LOG_{10} CFU/ML	LOG_{10} REDUCTION FROM INOCULUM (POSITIVE CTRL)	*P* VALUE FROM INOCULUM
Clostridioides difficile spores (ATCC 43598)	0	7.7518 (0.4572)	7.5612	<1.00000[a]	>6.75 >6.56	.034
	200	7.5188 (0.7213)	7.0202	<1.00000[a]	>6.51 >6.02	.034
Mycobacterium terrae (ATCC 15755)	0	8.4014 (0.1738)	7.8971	<1.00000[a]	>7.40 >6.90	.034
	200	8.2007 (0.1738)	7.9371	<1.00000[a]	>7.20 >6.94	.034
Escherichia coli (ATCC 11229) *Pseudomonas aeruginosa* (ATCC 15442) *Staphylococcus aureus* MRSA (ATCC 33592) *Klebsiella pneumoniae* (ATCC 10031)	0	8.1003 (0.1738)	7.9749	<1.00000[a]	>7.10 >6.97	.034
	200	9.2498 (0.1680)	7.9068	<1.00000[a]	>8.25 >6.91	.037

Abbreviations: cfu, colony-forming unit; MRSA, methicillin-resistant *Staphylococcus aureus*.
[a]No CFUs detected on any test barrier after laundry process.

Human studies

This study did not involve human participants.

Results

After laundering, no pathogenic microorganisms were detected on any of the test barriers (Table 2) or negative controls. No *C diff* spores were detected on new barriers or the 3 end-of-life barriers (>6.0 log_{10} cfu reduction). The 3 new barriers and the 3 end-of-life barriers inoculated with mixed pathogenic bacteria all had no detected bacteria (>6.9 log_{10} cfu reduction). The 3 new barriers and the 3 end-of-life barriers that had been contaminated with *M terrae* all had no detected bacteria (>6.9 log_{10} cfu reduction).

Each suspension was tested prior to inoculation onto the test barrier, and there were greater than 7.5 log_{10} cfu/mL of each of the microorganisms in the suspension. All positive controls had greater than 7.0 log_{10} cfu of microorganisms in the test area, and all negative controls had no detectable growth after laundering.

Discussion

The results indicate a commercial laundry process can remove all pathogenic bacteria and *C diff* spores from the barrier. This represents greater than 6 log_{10} reduction in microorganisms, which is far greater than the 1 to 2 log_{10} cfu reductions seen with manual cleaning using chemical disinfectants.

The laundry process provides detergent, bleach, agitation, and repeatability. These elements allow bacteria and spores to be physically separated from the barrier surface. The chlorine works to kill residual organisms. Multiple rinse cycles allow the microorganisms to be removed from the washing machine. Further study could determine the contribution of these various elements to the end result.

The manual process of cleaning hospital mattresses and beds used in most hospitals is typically a 1-step process, despite being off-label use of the disinfectant and the manufacturer's multistep instructions for cleaning and disinfection. It is not surprising this process fails to reduce the bacterial counts more than 1-2 log_{10} cfu.[6-11] Multiple studies have shown that if the previous patient had been infected, or even colonized with bacteria, there is a marked increased risk of the patient getting an HAI.[23-25] Mattress covers are soft hygroscopic materials, and not only will a 1-step process fail to disinfect, it may also leave residual chemical on the surface and damage the material. Many mattress manufacturers recommend a multistep cleaning process, including precleaning, cleaning with mild detergent, rinsing, disinfecting, rinsing again, and inspecting.[26-28] Multiple steps can result in improved cleaning and disinfection and protect the mattress cover material by removing the chemical disinfectant. However, a multistep process with rinses can quadruple bed reprocessing time compared with the 1-step process. In the United States, many hospitals routinely maintain occupancy rates close to 100%, which puts pressure on the environmental services workers to clean the room quickly.

The results of this study must be interpreted in light of its limitations. The study was relatively small sample size. Only 3 barriers were used for each condition (new and after 200 launderings). However, all 6 test barriers had no residual bacteria or

spores on them after the laundry process. This study uses a specific laundering process. Different laundry processes and chemicals may yield different results.

Conclusions

In this investigation, laundering using a commercial washer/extractor was able to remove all pathogenic bacteria and *C diff* spores from a barrier. This may offer a better method to prevent HAIs than currently recommended multistep process of manual cleaning of the mattress and bed deck.

Acknowledgments

Research was performed at Q Laboratories in Cincinnati, Ohio, USA.

Author Contributions

DU planned the study. EAH performed the statistical analyses. All authors read and approved the final manuscript.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ORCID iD

Edmond Anderson Hooker https://orcid.org/0000-0001-7307-9483

REFERENCES

1. Centers for Disease Control and Prevention. *Data Portal: Healthcare-Associated Infections: Data Highlights From HAI Hospital Prevalence Survey*. Centers for Disease Control and Prevention. https://www.cdc.gov/hai/data/portal/index.html. Published October 29, 2018. Accessed November 11, 2018.
2. Hand Hygiene in Healthcare Settings. Centers for Disease Control and Prevention. https://www.cdc.gov/handhygiene/index.html. Published May 3, 2018. Accessed November 11, 2018.
3. Creamer E, Humphreys H. The contribution of beds to healthcare-associated infection: the importance of adequate decontamination. *J Hosp Infect*. 2008;69:8-23.
4. Pantel A, Richaud-Morel B, Cazaban M, Bouziges N, Sotto A, Lavigne J-P. Environmental persistence of OXA-48–producing Klebsiella pneumoniae in a French intensive care unit. *Am J Infect Control*. 2016;44:366-368.
5. Bousquet A, van der Mee-Marquet N, Dubost C, et al. Outbreak of CTX-M-15–producing *Enterobacter cloacae* associated with therapeutic beds and syphons in an intensive care unit. *Am J Infect Control*. 2017;45:1160-1164. http://www.sciencedirect.com/science/article/pii/S0196655317303267. Accessed August 14, 2017.
6. Hooker EA, Allen SD, Gray LD. Terminal cleaning of hospital bed mattresses and bedecks does not eliminate bacterial contamination. *Am J Infect Control*. 2011;39:E23-E24.
7. Sigler V, Hensley S. Persistence of mixed staphylococci assemblages following disinfection of hospital room surfaces. *J Hosp Infect*. 2013;83:253-256.
8. Doan L, Forrest H, Fakis A, Craig J, Claxton L, Khare M. Clinical and cost effectiveness of eight disinfection methods for terminal disinfection of hospital isolation rooms contaminated with Clostridium difficile 027. *J Hosp Infect*. 2012;82:114-121.
9. Deshpande A, Mana TS, Cadnum JL, et al. Evaluation of a sporicidal peracetic acid/hydrogen peroxide —based daily disinfectant cleaner. *Infect Control Hosp Epidemiol*. 2014;35:1414-1416.
10. Liscynesky C, Hines LP, Smyer J, Hanrahan M, Orellana RC, Mangino JE. The effect of ultraviolet light on clostridium difficile spore recovery versus bleach alone. *Infect Control Hosp Epidemiol*. 2017;38:1116-1117.
11. Anderson DJ, Chen LF, Weber DJ, et al. Enhanced terminal room disinfection and acquisition and infection caused by multidrug-resistant organisms and Clostridium difficile (the Benefits of Enhanced Terminal Room Disinfection study): a cluster-randomised, multicentre, crossover study. *Lancet*. 2017;389:805-814.
12. Hooker E, Leigh Jones K. Cleaning practices for hospital mattresses in top US adult hospitals. *Am J Infect Control*. 2012;40:e43.
13. Bradbury SL, Mack D, Crofts T, Ellison RT 3rd. Potential bloodborne pathogen exposure from occult mattress damage. *Am J Infect Control*. 2014;42:421-422. doi:10.1016/j.ajic.2013.10.011.
14. Marks B, Abboud T. Uncovering the rate of damaged mattress covers in acute care hospitals. *Am J Infect Control*. 2016;44:S79.
15. Food and Drug Administration. *FDA Safety Communications: Damaged or Worn Covers for Medical Bed Mattresses Pose Risk of Contamination and Patient Infection*. Food and Drug Administration. https://wayback.archive-it.org/7993/20170722215739/https:/www.fda.gov/MedicalDevices/Safety/AlertsandNotices/ucm348016.htm. Accessed May 5, 2020.
16. *ECRI Institute's Top 10 Patient Safety Concerns for Healthcare Organizations 2018*. ECRI Institute. https://www.ecri.org/components/HRC/Pages/HRCWebinar_062118_Top10.aspx. Published 2018. Accessed October 30, 2018.
17. Hooker EA, Bochan M, Reiff TT, Blackwell C, Webb KW, Hart KW. Decreasing *Clostridium difficile* health care–associated infections through use of a launderable mattress cover. *Am J Infect Control*. 2015;43:1326-1330.
18. U.S. Environmental Protection Agency, Office of Pesticide Programs. *EPA MLB SOP MB-28: Procedure for the Production and Storage of Spores of Clostridium difficile for Use in the Efficacy Evaluation of Antimicrobial Agents*. https://www.epa.gov/sites/production/files/2017-09/documents/mb-28-final.pdf. Published September 2017. Accessed February 23, 2020.
19. Hucker GJ. Progress Report on Detergent Evaluation Investigations. New York State Agricultural Experiment Station; April 1, 1942-March 1, 1943; Geneva, NY.
20. Miles RS. What standards should we use for the disinfection of large equipment. *J Hosp Infect*. 1991;18:264-273.
21. U.S. Environmental Protection Agency, Office of Pesticide Programs. *EPA MLB SOP-MB-31: Procedure for the OECD Quantitative Method for Testing Antimicrobial Products against Spores of Clostridium difficile (ATCC 43598) on Inanimate, Hard, Non-Porous Surfaces*. https://www.epa.gov/sites/production/files/2017-09/documents/mb-31-final.pdf. Published September 2017. Accessed February 23, 2020.
22. ASTM International. *ASTM E1054-08, Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents*. West Conshohocken, PA: ASTM International.
23. Donskey CJ, Sunkesula VC, Stone ND, et al. Transmission of *Clostridium difficile* from asymptomatically colonized or infected long-term care facility residents. *Infect Control Hosp Epidemiol*. 2018;39:909-916.
24. Blixt T, Gradel KO, Homann C, et al. Asymptomatic carriers contribute to nosocomial Clostridium difficile infection: a cohort study of 4508 patients. *Gastroenterology*. 2017;152:1031.e2-1041.e2.
25. Russotto V, Cortegiani A, Raineri SM, Iozzo P, Gregoretti C, Giarratano A. What is the risk of acquiring bacteria from prior intensive care unit bed occupants? *Crit Care*. 2017;21:55.
26. Styker. *Stryker Operations/Maintenance Manual: IsoGel Air*. https://techweb.stryker.com/Support_Surfaces/2860/IsoGel/2860-009-001F.pdf. Accessed November 11, 2018.
27. Hill-Rom. *Compella™ Bariatric Bed System User Manual*. https://www.nhsggc.org.uk/media/239053/hillrom-compella-um.pdf. Accessed November 11, 2018.
28. Food and Drug Administration. *Reprocessing of Reusable Medical Devices: Information for Manufacturers*. https://www.fda.gov/medicaldevices/deviceregulationandguidance/reprocessingofreusablemedicaldevices/default.htm. Accessed November 13, 2018.