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Major Article

Washer disinfector and alkaline detergent efficacy against *C. difficile* on plastic bedpans

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Background: *Clostridioides difficile* is a major cause of infectious antibiotic resistant diarrhea. *C. difficile* spores are shed in patient stool, are hearty and difficult to kill. Bedpans are often used by patients with *C. difficile* infections and require proper handling and cleaning or disposal to prevent the transmission of *C. difficile* spores and other infectious microorganisms into the environment. Disposable bedpans are often used for convenience, which has consequences from an environmental sustainability perspective.

Aim: This study evaluates the ability for a washer-disinfector device (WD) to efficaciously clean and disinfect *C. difficile* spores and *Escherichia coli* from bedpans for sanitary reuse.

Methods: A commercially available WD device was evaluated for both efficacy and thermal disinfection against *C. difficile* spores and *Escherichia coli* using one disinfection cycle per test. Bedpans were not rinsed or dumped prior to placement in the WD. Bedpans were sampled using swabs. Microorganisms were eluted from the swabs and log-kill was calculated.

Findings: The average log-kill for *C. difficile* spores was 3.99 and >7.69 for *E. coli*. Thermal disinfection results showed an average log kill of 4.31 for *C. difficile* and >7.23 for *E. coli*.

Conclusions: The WD was efficacious against both *C. difficile spores* and *E. coli* when used according to manufacturer's instructions for use, suggesting a viable alternative to disposable bedpan waste management.

Clostridioides difficile (formerly known as *Clostridium difficile*) is colloquially referred to as *C. diff* and is an anaerobic, Gram positive, spore-forming bacterium.[1-4] *C. difficile* infection (CDI) can result in severe diarrhea and can progress to life threatening pseudomembranous colitis in some cases.[4] *C. difficile* is typically transferred from fecal sources and patients with CDI are a significant reservoir of *C. difficile* spores. The spore-forming ability of this bacterium makes it particularly resilient.[3,5]

C. difficile spores are much more persistent against common disinfectants when compared to their vegetative state.[6,7] In a study of healthcare workers dealing with CDI patients, 24% of the workers' hands were found to be contaminated with spores. In addition to direct contact with CDI patients, spores were also found to be transmitted to healthcare workers through surfaces, including bedrails, countertops, and sinks.[8] Healthcare worker exposure to items such as bedpans is an important consideration for the reduction of CDI.

Bedpan use is most frequent in areas where patient mobility is limited such as in long-term care facilities and acute care hospitals. Facilities may use reusable plastic bedpans that are cleaned between uses or single-use disposable bedpans. While reusable bedpans can reduce the solid waste and waste management concerns for a healthcare facility compared to single-use products, effective cleaning and disinfection is critical to limit pathogen exposure.[9] For facilities that use reusable bedpans, automated cleaning of bedpans and other equipment is typically performed by a washer-disinfector device (WD) at a central cleaning facility.[10,11] Alarmingly, effective cleaning of bedpans was found to be extremely limited in a study of 36 American hospitals,

Abbreviations: WD, washer disinfector device; CDI, *Clostridioides difficile* infection

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where compliance to cleaning regimens ranged between 0% and 79%.[12] WDs can be an effective alternative for reducing the workload required by cleaning staff and provide a consistent and surveillable method for the cleaning of soiled bedpans to enable safe reuse. In this study, the cleaning efficacy and thermal disinfection capacity of a commercially available WD is evaluated.

MATERIALS AND METHODS

Bedpan washing device

A commercially available bedpan WD (Meiko TopLine 20, LaVergne, TN) was evaluated using the "Intensive Program" cycle. The run cycle consists of a cold water wash then a warm water wash, followed by disinfection with steam from the internal steam generator to reach the disinfection parameter Ao-value of 60. The Ao-value is a combination of time and temperature. The maximum reached temperature inside the device is approximately 88°C. The cycle time is approximately 9 minutes to complete washing, disinfecting, and drying. Warm water wash includes a dosing amount of 7 g of alkaline detergent (Meiko, Doyen R100, 5 L). All testing was performed in the NSF International microbiology laboratory, Ann Arbor, Michigan.

Strains, media, and reagents

Clostridioides (formerly *Clostridium*) *difficile* ATCC 43598 and *Escherichia coli* ATCC BAA 2326 were purchased from American Type Culture Collection (Manassas, VA).

C. difficile was cultured and enumerated with CDC Anaerobe 5% Sheep Blood Agar (CABA; Becton Dickinson and Company, Sparks, MD), Reinforced Clostridial Medium (RCM; Becton Dickinson and Company, Sparks, MD), and Brain Heart Infusion Agar with Horse Blood & Taurocholate (BHIY-HT; Anaerobe Systems, Morgan Hill, CA). AnaeroPack Anaero sachets (Mitsubishi Gas Chemical, Tokyo, Japan) were used to maintain anaerobic conditions during incubation of media. Dilutions were performed with phosphate buffered saline (pH 7.2).[13]

E. coli was cultured and/or enumerated with Typticase Soy Broth (TSB; Becton Dickinson and Company, Sparks, MD), Levine eosin methylene blue (LEMB) agar (LEMB) (Criterion, Hardy Diagnostics, Santa Maria, CA), and Petrifilm *E. coli*/Coliform Count Plates (Petrifilm EC; 3M, St. Paul, MN). Dilutions were performed in phosphate buffer (3M, St. Paul, MN).

Unless otherwise stated, chemical reagents were purchased from Amresco (Salon, OH), BDH VWR Analytical (Radnor, PA), Fisher (Fair Lawn, NJ), and Sigma-Aldrich (St Louis, MO). Corn starch used in the preparation of test soil was manufactured by Argo (ACH Food Company, Inc., Memphis, TN).

Test soil and inoculum preparation

Test soil was prepared according to Annex H of ISO/TS 15883-5, excluding *Enterococcus faecium*, and consisted of (g/L): porcine mucin, 7.5; bovine albumin, 4.5; and corn starch, 45.[13] Test soil used in *C. difficile* and *E. coli* testing was prepared in distilled and ultrapure water, respectively. Temperature was not monitored during soil preparation.

C. difficile spores were prepared according to previously described methods with minor modifications.[14] A culture of RCM incubated at 36° ± 1.0°C for 24 ± 2 hours was used to inoculate CABA plates (0.1 mL/plate). After 10 days of incubation at 36° ± 1.0°C spores were harvested from the CABA plates using PBS-T, a phosphate buffered solution with 0.05% (v/v) Tween 20 (pH 7.4), washed 3 times by centrifugation (4,500 × *g* for 15 minutes), and resuspension in PBS-T. The spores were heated to 65° ± 2.0°C for 10 ± 1 minutes, cooled, then purified using density gradient centrifugation (4,500 × *g* for 15 minutes) with 50% HistoDenz (5 mL overlayed with 1 mL spore suspension, scaled as needed). The pellet was resuspended and washed 3 times (16,000 × *g* for 2-5 minutes) in cold PBS-T. The final PBS-T spore suspension was stored at or below −70°C prior to use. Spore purity was confirmed to be ≥95% by endospore staining with Malachite Green and Safranin. Spore quality was confirmed using a carrier-based test employing 2 concentrations of sodium hypochlorite (NaOCl).[15-17]

E. coli was grown in TSB at 34°-37°C for 18-24 hours, concentrated by centrifugation (4,750 × *g*, 40 minutes, 10°C) and resuspended in phosphate buffer.

Bedpan test

Bedpans (Medegen Stackable 2qt. Bedpan) were disinfected with 70% ethanol before testing. The test soil (0.1 mL) was combined with 0.1 mL *C. difficile* spore suspension or 0.02 mL *E. coli* suspension and applied to each bedpan once on the inside bottom (bowl) surface and once on the rim surface. Each inoculated area was approximately 100 cm^2 in size. Bedpans were dried for ≥5 hours under ambient temperature and humidity conditions then exposed to treatment by the automated bedpan WD. Eight treated bedpans and 4 untreated (control) bedpans were employed in each organism test. Two control bedpans were inoculated and sampled in parallel with the first treated bedpan and 2 in parallel with the last treated bedpan.

Inoculated areas were swabbed with 3M Enviro swabs (3M, St. Paul, MN) by applying 25 swabbing movements horizontally, vertically, and diagonally. For *C. difficile* testing, swabs was transferred to a Coy Vinyl Anaerobic Chamber (COY Laboratory Products, Grass Lake, MI) maintained with an atmosphere of <0.03% O_2, 1.6%-4.0% H_2, and balanced with N_2. Each swab was eluted in 10 mL *Clostridium difficile* Banana Broth (Hardy Diagnostics) or phosphate buffer for *C. difficile* and *E. coli*, respectively, and vortexed for 30 seconds. Swab eluents were diluted and applied in duplicate to BHIY-HT agar plates (*C. difficile*) and Petrifilm EC Plates (*E. coli*) and incubated at 36° ± 1° C for 48 ± 4 hours.

Thermal disinfection test

For *C. difficile* testing, the test soil (0.9 mL) was combined with 0.1 mL *C. difficile* spore suspension in sterile screw-cap cryovials and mixed by vortexing. For *E. coli* testing, 0.975 mL soil was combined with 0.025 mL cell suspension. One hundred microliters were drawn from each vial before treatment and reserved for enumeration. The vials were exposed to treatment by the automated bedpan WD (one vial per cycle). Four vials were employed in each organism test. Challenge organism was enumerated on BHIY-HT agar plates and Petrifilm EC Plates for *C. difficile* and *E. coli*, respectively.

Statistical analysis

The paired t test and Mann Whitney comparative tests were performed using GraphPad Prism 7 for Windows, version 7.00 (1992-2016 GraphPad Software, Inc.).

RESULTS

In the first set of experiments, the average logarithmic reduction of *E. coli* in a bedpan was reduced by more than 7.69 (Table 1). In a reciprocal experiment, *C. difficile* spores were logarithmically reduced by 3.99. Both changes in recoverable bacteria were statistically significant ($P < .0001$ and $P < .0001$, respectively).

The automated bedpan WD system was further evaluated with their ability to thermally inactivate or decontaminate the *E. coli* and

Table 1
Average bioburden reduction of *C. difficile* and *E. coli* on bedpans following treatment with the washer-disinfector device. The limit of detection for the bedpan culture testing was 100 and 10 CFU/site for *C. difficile* and *E. coli*, respectively

	Control (Log10 CFU/site)	Test (Log10 CFU/site)	Average log reduction
E. coli	8.71	<1.02	>7.69
C. difficile	7.68	3.7	3.99

Table 2
Average bioburden reduction of *C. difficile* and *E. coli* in cryovials following treatment with the bedpan washer-disinfector device. The limit of detection for the cryovial culture testing was 1 CFU/mL for both organisms

	Control (Log10 CFU/mL)	Test (Log10 CFU/mL)	Average log reduction
E. coli	8.23	<1.0	>7.23
C. difficile	8.54	4.23	4.31

C. difficile cultures (Table 2). The thermal capacity of the WD was found to be capable of causing a greater than 7.23 and 4.31 logarithmic reduction, respectively. Both reductions in culturable bacteria were statistically significant ($P < .001$ *E. coli* and $P = .001$ *C. difficile*).

DISCUSSION

Disinfection with the WD demonstrated a statistically significant reduction in both *E. coli* and *C. difficile* soiled samples following one cleaning cycle ($P < .0001$ and $P < .0001$, respectively). The decrease in number of culturable *E. coli* was greater on the bedpan, which showed a greater than 7.69 logarithmic reduction, but the results of the thermal disinfection test showed that much of the reduction can be attributed to the temperature to which the sample is subjected. *C. difficile* is notoriously resilient against heat disinfection, and typically requires temperatures greater than 85°C for sufficient thermal disinfection.[18] For *C. difficile* in the bedpan, the WD had a 3.99 logarithmic reduction after one cycle. Unlike *E. coli*, the thermal disinfection test increased the logarithmic reduction of the spores to 4.31. Given our knowledge of *C. difficile* spores and their heartiness against most cleaning methods, it is important to note that high temperatures are an important component of the decontamination of medical equipment.[18]

Previous studies have shown that the presence of soil on surfaces can improve survival of microorganisms by limiting their exposure to the disinfectants.[10,19] Organic soiling represents an accumulation of mostly human compounds that may build up on surfaces. We can simulate such soiling by adding proteins or mixed compound solutions into the bacterial inoculum. By including organic soils, a more accurate examination of the WD's real-world use can be assessed since microorganisms are rarely transferred to surfaces in isolation. On bedpans, the soil can form a physical barrier that isolates the microorganisms from the disinfectant or physical removal of the bacteria. Despite the additional barriers against cleaning from the organic soiling, the WD still provided a statistically significant reduction of bacterial load on the bedpan. *C. difficile* reduction was found to be comparable to previous studies of other WD systems.[11,20] In a study of cleaning efficacy, direct washing of bedpans contaminated with spores revealed a >4.65 logarithmic reduction.[11] A similar study that evaluated WD with different detergents showed a >5.95 logarithmic reduction, which suggests that detergent selection, as well as cleaning cycle are important factors for the elimination of *C. difficile* spores. In those same studies, cryovial logarithmic reduction rates were at least >3.243 (depending on the detergent), and >1.12, depending on the testing site, respectively.[11,20] It should be noted that these differences in logarithmic reductions were not strictly a result of the WD or the cleaning cycle settings, as the initial inocula in this study were higher (Log10 of 8.54 vs 4.6-5.7 and 5.95, respectively), and difference in soiling material can have an impact on results.[11,20]

The thermal disinfection efficacy was evaluated in the absence of a detergent and physical washing in the vial testing experiments. Similar studies have demonstrated the need for this type of validation, finding that some WDs did not reach sufficient temperatures and exposure times for the elimination of many bacteria, including spores.[10,11,20]

WDs are an effective tool and should be employed as part of a comprehensive cleaning regimen. For items that can be cleaned using heat sterilization, the use of sufficient heat to kill spores and other pathogens is recommended and should be instituted in central processing facilities.[11] When used appropriately, WDs can support the safe reuse of bedpans while limiting the transmission of pathogenic microorganisms.

Limitations

These experiments represent a controlled, *in vitro* study of an automated WD system. The study's experimental design relied on bacterial culture to quantify the reduction in the *E. coli* and *C. difficile* populations. Some bacteria may remain dormant, and fail to grow during culturing, but remain viable in optimal conditions. Additionally, it should be noted that the use of WDs can reduce the chance of contamination of healthcare workers and patients during bedpan and equipment cleaning, but it has little impact on other moments of potential contamination.[8,19] The results of this study may not be generalizable to other WD systems. The control settings and capability of each system should be evaluated to ensure that minimum temperatures needed for efficacy against *C. difficile* and other hardy bacteria are being met.

CONCLUSIONS

The speed, consistency, and sustainability achieved through the use of a WD for the cleaning and disinfection of reusable bedpans makes it an attractive alternative to manual cleaning methodologies or disposables. However, the capability of the WD with respect to efficacy must be a primary consideration. Although all bacterial spores were not eliminated, the automated WD tested in this study statistically significantly reduced bacterial spores (3.99 log10 reduction) and vegetative cells (>7.69 log10 reduction) on bedpan surfaces through standard cleaning as well as solely by thermal disinfection (4.31 and >7.23 log10 reduction, respectively). However, even with a high reduction of bacterial spores and vegetative cells, it is possible for residual bacteria to still be present on the bedpan. In order to determine the true return on investment, direct comparative studies assessing the impact that the WD has on reducing bacterial spores compared to manual cleaning are needed.

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References

1. Lawson PA, Citron DM, Tyrrell KL, Finegold SM. Reclassification of *Clostridium difficile* as Clostridioides difficile (Hall and O'Toole 1935) Prévot 1938. Anaerobe 2016;40:95-9.
2. Rupnik M, Wilcox MH, Gerding DN. Clostridium difficile infection: new developments in epidemiology and pathogenesis. Nat Rev Microbiol 2009;7:526-36.

3. Centers for Disease Control and Prevention (CDC). Vital signs: preventing *Clostridium difficile* infections. MMWR Morb Mortal Wkly Rep 2012;61:157-62.
4. Nagy E. What do we know about the diagnostics, treatment and epidemiology of Clostridioides (Clostridium) difficile infection in Europe? J Infect Chemother 2018;24:164-70.
5. Cohen SH, Gerding DN, Johnson S, et al. Clinical practice guidelines for clostridium difficile infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). Infect Control Hosp Epidemiol 2010;31:431-55.
6. Fawley WN, Underwood S, Freeman J, et al. Efficacy of hospital cleaning agents and germicides against epidemic *Clostridium difficile* strains. Infect Control Hosp Epidemiol 2007;28:920-5.
7. Wilcox MH, Fawley WN. Hospital disinfectants and spore formation by *Clostridium difficile*. Lancet 2000;356:1324.
8. Landelle C, Verachten M, Legrand P, Girou E, Barbut F, Buisson CB. Contamination of healthcare workers' hands with clostridium difficile spores after caring for patients with *C. difficile* Infection. Infect Control Hosp Epidemiol 2014;35:10-5.
9. Rutala WA, Gergen MF, Weber DJ. Efficacy of different cleaning and disinfection methods against clostridium difficile spores: importance of physical removal versus sporicidal inactivation. Infect Control Hosp Epidemiol 2012;33:1255-8.
10. Alfa MJ, Olson N, Buelow-Smith L. Simulated-use testing of bedpan and urinal washer disinfectors: evaluation of clostrium difficile spore survival and cleaning efficacy. Am J Infect Control 2008;36:5-11.
11. Alfa MJ, Olson N, Buelow-Smith L, Murray B-L. Alkaline detergent combined with a routine ward bedpan washer disinfector cycle eradicates *Clostridium difficile* spores from the surface of plastic bedpans. Am J Infect Control 2013;41:381-3.
12. Carling PC, Parry MM, Rupp ME, et al. Improving cleaning of the environment surrounding patients in 36 acute care hospitals. Infect Control Hosp Epidemiol 2008;29:1035-41.
13. EPA MLB SOP MB-28: Procedure for the production and storage or spores of *Clostridium difficile* for use in the efficacy evaluation of antimicrobial agents. 2017. Available at: https://www.epa.gov/sites/production/files/2017-09/documents/mb-28-final.pdf. Accessed December 23, 2019.
14. EPA MLB SOP MB-31: Quantitative method for testing antimicrobial products against spores of *Clostridium difficile* (ATCC 43598) on inanimate, hard, non-porous surfaces. 2017. Available at: https://www.epa.gov/sites/production/files/2017-12/documents/mb-31_december_2017.pdf. Accessed December 23, 2019.
15. Jullian-Desayes I, Landelle C, Mallaret M-R, Brun-Buisson C, Barbut F. *Clostridium difficile* contamination of health care workers' hands and its potential contribution to the spread of infection: review of the literature. Am J Infect Control 2017;45:51-8.
16. International Organization for Standardization. Washer-Disnifectors - Part 5: Test Soils and Methods for Demonstrating Cleaning Efficacy. (ISO/TS 15883-5). Geneva: ISO; 2005.
17. Rodriguez-Palacios A, Lejeune JT. Moist-heat resistance, spore aging, and superdormancy in clostridium difficile. Appl Environ Microbiol 2011;77:3085-91.
18. Verran J, Whitehead KA. Assessment of organic materials and microbial components on hygienic surfaces. Food Bioprod Process 2006;84:260-4.
19. Zühlsdorf B, Winkler A, Dietze B, Floss H, Martiny H. Gastroscope processing in washer–disinfectors at three different temperatures. J Hosp Infect 2003;55:276-82.
20. MacDonald K, Bishop J, Dobbyn B, Kibsey P, Alfa MJ. Reproducible elimination of clostridium difficile spores using a clinical area washer disinfector in 3 different health care sites. Am J Infect Control 2016;44:e107-11.