Author copy. The definitive article may be found at Am J Infect Control **American Journal of Infection Control 46 (2018) 1154-9**). https://doi.org/10.1016/j.ajic.2018.04.206.

A microbiological study to investigate the carriage and transmission-potential of *Clostridium difficile* spores on single-use and reusable sharps containers

Terry Grimmond FASM, BAgrSc, GrDpAdEd&Tr[a], terry@terrygrimmond.com **Corresponding author**
Anu Neelakanta MD MPH[b],
Barbara Miller MSN, BSN, CPSO, CHSP[c],
Asif Saiyed MT (AMT), BS, BA, MBA, CIC[d],
Pam Gill MHA(c), BSN, BBA, RN, CIC, CRNI[e],
Jennifer Cadnum BS[f],
Russell Olmsted MPH, CIC, FAPIC[g],
Curtis Donskey MD[h],
Kimberly Pate MSN, RN, ACCNS-AG, PCCN[i],
Katherine Miller BS[j].

[a] Director, Grimmond & Associates, Microbiology Consultants, Hamilton, New Zealand.
[b] Department of Infectious Diseases, Carolinas Medical Center, Charlotte, NC
[c] Environmental Health and Safety Dept., Carolinas Health System, Charlotte, NC
[d] Infection Control, Sinai Health System, Chicago, IL
[e] Infection Prevention, Iredell Health System, Statesville, NC
[f] Research Services, Louis Stokes Cleveland Veterans Affairs Medical Center, Cleveland, OH
[g] Infection Prevention & Control, Trinity Health Unified Clinical Organization, Livonia, MI
[h] Infection Control Department, Louis Stokes Cleveland Veterans Affairs Medical Center, Cleveland, OH; and Research Services, Louis Stokes Cleveland Veterans Affairs Medical Center, Cleveland, OH
[i] Surgical-Trauma Division, Carolinas Medical Center, Charlotte, NC
[j] Levine Cancer Institute, Carolinas HealthCare System, Charlotte, NC.

Funding/Support: Investigation was supported by Daniels Health and included costs associated with conducting the study and use of processing facilities to randomly sample reusable sharps containers and conduct challenge tests. **Disclaimer**: Daniels Health was not involved nor reviewed details of study design, collation, results, analysis, or study manuscript.

ABSTRACT

Background. A 2015 study matching use of disposable and reusable sharps containers (DSC; RSC) with *C. difficile* infection (CDI) incidence found a decreased incidence with DSC. We conducted microbiological samplings and examined the literature and disease-transmission principles to evaluate the scientific feasibility of such an association.

Methods. (i) 197 RSC were sampled for *C. difficile* at processing facilities; (ii) RSC were challenged with high *C. difficile* densities to evaluate efficacy of automated decontamination; (iii) 50 RSC and 50 DSC were sampled in CDI patient rooms in 7 hospitals. Results were coupled with epidemiological studies, clinical requirements and Chain-of-Infection principles, and Tests-of-Evidence of disease transmission applied.

Results. *C. difficile* spores were found on 9 of 197 (4.6%) RSC prior to processing. Processing completely removed *C. difficile*. In CDI patient rooms, 4 of 50 RSC (8.0%) and 8 of 50 DSC (16.0%) had sub-infective counts of *C. difficile* (p=0.27). DSC were in permanent wall cabinets; RSC were removed and decontaminated frequently.

Conclusion. With *C. difficile* bioburden being sub-infective on both DSC and RSC, sharps containers being no-touch, and glove-removal required after sharps disposal, we found two links in the Chain of infection to be broken and 5 of 7 Tests of Evidence to be unmet. We conclude sharps containers pose no risk of *C. difficile* transmission.

Key words: Environment, Hospital, Chain of Infection, Reservoir, Infective dose, Waste bin, Clinical practice, Disposable.

BACKGROUND

In 2011, *Clostridium difficile* (CDI) was responsible for 453,000 health care–associated infections (HAI) and 29,000 deaths in U.S. hospitals.[1] Despite an 8% decrease in incidence by 2014,[2] it is the most commonly reported pathogen causing HAI.[3]

C. difficile is spread by the fecal-oral route and ~~a~~ny surface, device, or material contaminated with feces containing *C. difficile* vegetative cells or endospores may serve as a reservoir for this pathogen.[4] Hospitalised, non-colonized patients may acquire *C. difficile* directly from other patients with CDI or indirectly from the environmental or from contaminated hands of healthcare personnel.[5] Surfaces in the patient room environment serve as a reservoir for 2-10% of CDI cases,[6-9] and frequently touched surfaces close to the patient's bed are more likely the source of *C. difficile* contamination.[10-13]

Sharps containers are an "environmental surface" and may be a disposable (single-use) sharps container (DSC) or a reusable sharps container (RSC) and in the U.S. have close to an equal market share. In patient rooms they are commonly wall-mounted 1.5-3m from the patient's bed. Although attention has been drawn to the microbial bioburden of poorly-cleaned reusable regulated medical waste bins[14] and reusable sharps containers,[15] neither DSC or RSC have been scientifically shown to be a fomite. With specific reference to CDI, neither DSC nor RSC have been mentioned as a "touched-item" in *C. difficile* environmental surveys,[10-13,16] nor as a potential fomite in *C. difficile* evidence-based prevention guidelines.[6,17,18]

However, in 2015, Pogorzelska-Maziarz obtained hospital-wide CDI rates via Medicare Provider Analysis and Review data, ascertained and linked RSC or DSC usage via a telephone questionnaire of participating hospitals, and found a significantly lower rate of CDI in hospitals using DSC over those using RSC (Incidence Risk Ratio = 0.846, p = 0.001).[19] Dikon questioned the scientific credibility of the survey,[20] and Pogorzelska-Maziarz in reply confirmed the soundness of the methodology[21] but did not propose a scientific explanation as to how sharps containers might act as a fomite, and recommended further studies involving direct culturing of sharps containers.

We elected to conduct such a study - our hypothesis being that the statistical association found by Pogorzelska-Maziarz study is an artifact and that sharps containers (irrespective of type) may have a low spore burden but have no fomite potential. We adopted a multi-faceted epidemiological, microbiological, Chain of Infection and Test of Evidence approach to determine if a relationship between CDI and sharps containers was scientifically feasible.

METHODS

The microbiological aspect consisted of three stages and was approved by institutional review boards at participating hospitals. An RSC company (Daniels Health, Chicago IL) was approached and permission obtained to sample recently-received full RSC before processing at their factories (Stage 1) and to conduct the challenge experiments (Stage 2). Six multi-hospital systems using RSC and/or DSC were contacted by the authors and invited to participate in Stage 3. An equal total number of beds was sought for RSC and DSC hospitals.

Stage 1 – Do RSC carry C. difficile upon arrival at processing facilities?

One hundred and ninety-seven, full, used, 22-liter RSC (Sharpsmarts, Daniels Health, Chicago IL) received at four geographically unique processing facilities (Sturtevant WI, Walton KY, Westland MI and Fresno CA) were randomly chosen on arrival from client hospitals. Prior to processing, the RSC were swabbed for presence of *C. difficile* by the same microbiologist at each processing facility. BD BBL Culture Swab Collection and Transport System (BD, Franklin Lakes, NJ) were used and each sterile package contained two swabs co-joined by a cap, and a sealed tube with transport medium in its base. Using a thumb-forefinger rolling action, a dual swab-set moistened in sterile water was briskly rubbed in two opposing directions over the entire front surface and lid (approximately 700 cm^2 in total), inserted into labelled transport medium and couriered to a *C. difficile* reference laboratory at VA Medical Center, Cleveland OH. At the lab, each swab was cultured for broth enrichment into a tube of *C. difficile* Brucella Broth with thioglycolic acid and L-cystine (CDBB-TC) (qualitative growth) and incubated aerobically at 37^0C for 72 hours[22] (CDBB-TC was chosen due to its superior sensitivity and specificity over standard C. difficile growth media[22]). *C. difficile* was confirmed by morphology and Microgen latex agglutination (Microgen Bioproducts, Surrey, UK). Culture results for this stage were recorded as Pos/Neg. Quantitation of *C. difficile* spores (via direct plate culture) was not conducted in Stage 1. Isolates were tested for toxin production by sub-culturing the isolate into 1 mL of

Brucella broth, incubating anaerobically for 3 days (to accumulate toxin) then conducting a toxin assay on the broth using a commercial kit (Alere Inc., Waltham, MA). The sterile water used at each factory and an unopened swab were submitted for culture as negative controls. The results of Stage 1 were used to calculate sample sizes for Stage 3.[23]

Stage 2 **– Does the decontamination process remove *C. difficile* spores from RSC?**

The proprietary decontamination process of the RSC in this study comprised 6 wash stages: two cold water flushes, two hot water washes at 55^{0}C (the first with detergent), a scald rinse with water at 85^{0}C, an air-knife drying stage, and a final stage where a fine film of a proprietary formulation was applied to all internal surfaces to decrease adherence of organic matter and soil during the next client's use. The process has been repeatedly validated by independent laboratories to achieve a 6-log reduction of vegetative pathogens and a 4 log reduction of spores. Our experience in patient room surface-sampling indicated that the density and frequency of C. difficile spores on RSC would be low given they are no-touch[20] and are distant from the patient.[24,25] A statistical power calculation indicated, that with low density and frequency, several hundred RSC would need be microbiologically sampled after processing. Instead we elected to increase the test-severity by challenging the process's ability to remove high-density challenges of *C. difficile* spores applied to RSC. Our reference laboratory standardly conducts *C. difficile* surface-challenges in two x triplicate tests (6 surfaces) however we increased this number to 10 RSC. The challenge suspension of nontoxigenic spores (in sterile water) was supplied by the *C. difficile* reference laboratory and was designed to apply *C. difficile* spores to RSC at two densities: a high-density at approximately 1,000 to 10,000-fold higher than counts we standardly find on low-touch CDI patient room surfaces; and a medium density of 1/100 dilution of the high-density suspension. Using a sterile transfer-pipette, one drop (approximately 0.2 ml) of high-density suspension was applied in a precise area to the right side of the counterbalanced tray on four clean RSC and a second drop (Positive control) placed on the left side. The medium-density suspension (test and control drops) were applied in the same manner in a defined area on the front of six clean RSC. Once dry, a 10 x 10 cm square area encompassing the control drop was swabbed in two opposing directions with a sterile moistened dual-swab and the labeled swab placed in its sterile transport media tube. The 10 RSC were subjected to the factory's automated decontamination process ("Washsmart"), according to the factory's standard protocol.

Upon completion of the process, for each of the 10 RSC, a 10 x 10 cm square area with the test-drop area in its centre was swabbed in the same manner as the control drop areas. All control and test swabs were couriered to the *C. difficile* reference lab. Each swab set was suspended in 200ul of sterile water and vigorously vortexed for 1 min. An aliquot was taken from the eluant and serially diluted and plated onto *C. difficile* Brucella Agar (CDBA) and incubated anaerobically at 37^{0}C for 72 hours and any resultant growth quantitated.[26]

Stage 3 - Do DSC and RSC in CDI patient rooms differ in their carriage rate of *C. difficile* spores?

In the rooms of all patients recently diagnosed with active CDI in hospitals using DSC or RSC, the containers had their outer surfaces swabbed in-situ for the presence of *C. difficile* spores at least 48 hrs after the diagnosis was made (to allow time for spore dispersal). Each CDI patient was involved only once. The sample size was determined by Stage 1 results.

Seven hospitals in North Carolina and Illinois using RSC or DSC (or both) participated in this stage. BD dual swabs were suppled to all hospitals and study personnel were instructed in the swabbing technique. Sharps containers were swabbed in the same manner as in Stage 1. If DSC were housed in cabinets, then the cabinet front and top were swabbed covering an area of approximately 700 cm^2. Both container types are of similar size and container and cabinet front vertical surfaces were included as they are the largest surface, and static charges on fomites may attract bacteria at a rate greater than gravitational sedimentation.[27] All swabs were refrigerated and couriered to the *C. difficile* reference lab where one swab was direct-cultured onto a CDBA plate and incubated anaerobically at 37^{0}C for 72 hours and colony forming units (cfu) counted, and the second swab cultured in CDBB-TC as in Stage 1. Details regarding sharps container cleaning protocols were sought from each facility.

Results were analyzed using WinPepi v11.26 (Abramson J, School of Public Health and Community Medicine, Hebrew University, Jerusalem, Israel). A Yates-corrected $\chi 2$ test was used for the analysis of proportions. If the cell value was <5 in the 2 × 2 table, Fisher's exact test was used. All P values were 2-sided. Statistical significance was set at $p \leq 0.05$.

RESULTS

In Stage 1, 9 of 197 (4.6%) RSC were positive by enrichment broth culture only. All controls were negative. Eight of the 9 isolates were toxigenic strains. Using the RSC positivity rate of 4.6% and a DSC rate 15% lower to reflect the IRR of Pogorzelska-Maziarz study, and to eliminate a type 1 error in Stage 3 (using 80% power and significance of 0.05), the sample size was determined to be 13,036 for both containers.

In Stage 2 the control swabs of the high-density and medium-density challenge-drops yielded an average of 4.44 Logs and 1.51 Logs of *C. difficile* spores respectively, equating to approximately 25,000 and 30 spores respectively. Following routine decontamination processing, all test-drop areas yielded zero spores (Table 1).

In Stage 3, 7 hospitals in 2 states participated. The sample size of 13,036 (determined in Stage 1) was deemed impractical as, in the 7 participating hospitals, it would require many decades to complete. In lieu, a pilot sampling of 50 RSC (from 2 hospitals, total 909 beds) and 50 DSC (from 5 hospitals, total 885 beds) was conducted over a 9-month period to obtain an estimate of the positivity rate in patient rooms for both containers. Samplings from CDI patients in varied clinical units (med-surg, ICU, rehab) were taken from 3-23 days after the CDI diagnosis was made (median 4.0 days). The length of time the container had been in the room prior to the CDI patient was not recorded. The samplings revealed a *C. difficile* carriage rate of 8.0% with RSC (all enrichment broth) and 16% with DSC (1 also positive on CDBA - 7 cfu) (Table 2). Although the frequency of carriage of *C. difficile* on RSC in CDI patient rooms was not significantly different from random RSC sampling in Stage 1 (4/50 vs 9/197; p = 0.33), this may be due to sample sizes however there was an 83% statistical chance that those from CDI patient rooms were higher.

All hospitals disinfected the external surfaces of wall-mounted RSC and DSC cabinets with either chlorine-based or hydrogen peroxide-peracetic acid disinfectants. Six hospitals did this daily and at patient discharge, and one hospital at patient discharge only.

Table 1. Stage 2 – efficacy of RSC decontamination process: average spore density (n^{10})

High-density Cd challenge (Log 10)		Medium-density Cd challenge (Log 10)	
Control area pre-wash	Test area post-wash	Control area pre-wash	Test area post-wash
4.44	0*	1.51	0*

*No spores were detected after the decontamination process.
RSC reusable sharps containers; Cd *C. difficile*

Table 2. Stage 3 – sampling of RSC and DSC in rooms of CDI patients#

	Number containers sampled	Number containers +ve for Cd in enrichment broth (%)	Number containers +ve for Cd on CDBA plates	Estimated highest spore count/cm^2
RSC	50	4 (8.0%)*	0	0.007
DSC	50	8 (16.0%)*	1	0.07

Sampling area = 700 cm^2 on RSC and DSC; *No significant difference (p = 0.22)
RSC reusable sharps containers; DSC disposable sharps containers; Cd *C. difficile*

DISCUSSION

Two articles prior to Pogorzelska-Maziarz have examined the microbiology of reusable bins.[14,15] Neely et al., disturbed that reusable medical waste bins (not sharps containers) were arriving visibly unclean, microbiologically examined reusable and disposable medical waste bins.[14] Both bin types had potential pathogens present with reusable having a higher rate than disposable (94% vs 10%). The authors instituted hospital-wide changes in room-cleaning, glove-use, handwashing, and reusable bin-cleaning and lid-handling, and found a 45% decrease in nosocomial infection rates over the 5-year study (p = 0.05). However, in acknowledging the wide range of changes implemented, the authors stated, *"…there is no direct proof that microorganisms from the infectious waste boxes caused nosocomial infections in patients"*.[14]

The second article,[15] conducted at a 130-bed community hospital, examined RSC that were

grossly unclean on arrival. The author found several organism types including gram-negative organisms and genomic remnants of bloodborne viruses in 3-13% of containers. The author stated RSC may be an infection risk to staff and patients and suggested further studies. Both studies offer a reminder that due diligence is essential in selecting a waste contractor with high standards of service and cleanliness, however neither study scientifically linked reusable bins with disease transmission.

The goal of our investigation was to scientifically investigate the potential for sharps containers to transmit an infective dose of *C. difficile* to patients.

The patient environment and *C. difficile* Chain of Infection

Patient-room surfaces may serve as a reservoir in the transmission of *C. difficile* spores to susceptible patients.[17,28] Determining which surfaces may be *C. difficile* reservoirs is essential in understanding cross-transmission[5] and is consistent with well-established principles in the Chain of Infection.[29] Specifically, each of the 6 links in the Chain (Pathogen; Reservoir; Mode of transmission; Portal of Entry; Susceptible host; Portal of exit) is essential for disease transmission – break one link and transmission stops.[30]

Contaminated, nearby, high-touch surfaces are associated with CDI transmission to patients,[10,31] confirming that a "Pathogen", "Portal of Entry", "Susceptible Host", and "Portal of Exit" are present. We therefore addressed the two remaining links ("Reservoir" and "Mode of Transmission") to investigate the possibility of a causal association between sharps containers and CDI.

Sharps containers as a reservoir of *C. difficile* spores

For a sharps container to serve as a reservoir (fomite), it must be contaminated with the pathogen at a sufficient density to constitute an infective dose.[32,33] Surfaces in the patient-room environment are a reservoir for *C. difficile* in 2-10% of CDI cases, [6-9] however not all surfaces have that capability. Of the 6 levels of infection risk that an instrument or surface may pose, low-touch environmental surfaces have the least risk.[33] Heavily-contaminated surfaces close to the patient are the common reservoirs for CDI transmission.[10,11,13,16,34-36]

Spores may be deposited on surfaces if they become airborne after being disturbed on other surfaces,[11] but airborne *C. difficile* spores commonly aggregate or attach to larger particles and fall to the floor relatively quickly.[24] Bathrooms excepted, environmental surfaces that are distant from the patient's bed are less frequently and less heavily contaminated than frequently-touched nearby surfaces (bed rails, bedside tables, call buttons, telephones, etc.) [12,24,25,37]

In *C. difficile* environmental-sampling studies, spores have been detected on a frequently-touched, wall-mounted glove dispenser,[38] but not on walls.[28,31,37] Walls (and wall-mounted fixtures) are considered non-critical surfaces,[33,39] as they *"...carry few microbes and those which are there are not readily detached."*[40] Furthermore, airborne dispersal to low-touch surfaces may be irrelevant in *C. difficile* transmission as these surfaces are infrequently touched.[25]

From the above epidemiology, wall-mounted sharps containers, being at a distance from the patient and not touched during use, would have little spore bioburden. Our Stage 3 results confirm this. The *C. difficile* spores found on the 4 patient-room RSC were detected only by enrichment CDBB-TC liquid culture (all CDBA plates negative). Research published by our reference laboratory found CDBB-TC +ve/CDBA -ve swabs have a *C. difficile* bioburden at the lower end of 1-10 spores.[22] Assuming a count of 5 spores, and a sampled area of 700 cm^2, the highest detectable spore bioburden on RSC was 0.007/cm^2. Of the 8 DSC found positive using enrichment broth, 1 CDBA grew 7 cfu indicating a spore-density at the lower end of 10-100 spores which, assuming a count of 50, equates to 0.07/cm^2.

Guerrero et al found the average number of colonies picked up by hands after touching high-touch, contaminated surfaces nearby to patients was 7 colony forming units (cfu) (range 1-60).[13] We calculated finger-tip surface area of one hand to be 17 cm^2 and if this finger-area picked up 7 spores then the spore density on the contaminated surface would be of the order of 0.4/cm^2, similar to the 0.3 spores/cm^2 found by others.[10,28] Lawley et al.,[41] found that the *C. difficile* infective dose in a murine model was 5-10 *C. difficile* spores/cm^2. Our swabbing technique has been shown to be an effective surrogate for hand pick-up of *C. difficile* spores from surfaces,[42] and these patient-room spore densities and infective doses are some 1000-fold higher than the density we found on RSC (0.007/ cm^2) or DSC (0.07/ cm^2).

For RSC to be causally-related to C. difficile transmission (given that design, shape, usage, patient-distance and fill-time of DSC and RSC are identical) RSC would need arrive at the healthcare facility with more *C. difficile* spores on their surfaces

than DSC. However this is not plausible as: In Stage 2 the decontamination process rendered RSC spore-free; clean and used RSC, if road-transported together, are separated; In Stage 3 patient-room RSC spore-density was extremely low - and no higher than DSC.

Our results show there is an insufficient number of spores on sharps containers to constitute an "infective dose" and thus sharps containers neither meet the epidemiological nor microbiological criteria for "reservoir". This breaks one link in the Chain and transmission cannot occur.

Sharps containers and Mode of Transmission of *C. difficile* spores

For sharps containers to be a mode of CDI transmission, spores must get from the sharps container to the patient. But how? Contaminated hands of staff (from touching heavily-contaminated surfaces close to the patient) are the common mode of transmission of *C. difficile* to patients.[10,17,18,28,31,43] However, sharps containers are designed for hands-free deposition of used sharps, i.e. the sharp is dropped into the aperture with one hand and neither the depositing hand nor the other hand touch the container.[20] Moreover, healthcare personnel must wear gloves while attending CDI patients,[6] and removal of gloves and hand hygiene is recommended after each patient interation,[17] and is mandatory after a sharps procedure with potential blood and body fluid exposure.[44, 45] With either of these two practices (hands-free deposition; removal of gloves) a "mode of transmission" to a patient is not possible. This breaks a second link in the Chain and transmission cannot occur.

RSC vs DSC in relation to *C. difficile* transmission

Pogorzelska-Maziarz found a statistical link between use of single-use sharps containers and lower *C difficile* case-rates.[19] We do not dispute Pogorzelska-Maziarz's finding but we find it problematic to support the assertion that sharps containers may serve as a fomite. Disposable and reusable sharps containers are similar in design, shape, function, usage, placement, cleaning while in use, fill-time, and hospital handling. They differ only in that wall-mounted DSCs commonly have an external cabinet which remains permanently on the wall, while the RSC in this study were not housed in cabinets and were regularly removed for decontamination (with other RSC brands, they too are removed regularly but are commonly housed in cabinets identical to DSC). Our finding of a higher, but statistically non-significant, carriage rate of *C. difficile* spores on DSC cabinets (16.0% vs 8.0% in RSC; p = 0.22) is likely due to the pilot-sample size being lower than that indicated in stage 1, however it was deemed adequate for this study as: (i) the statistical analysis revealed a 90% chance that the higher carriage incidence of *C. difficile* on DSC was true; (ii) a second power calculation using Stage 3 carriage rates indicated that 552 samples of each container would be required to achieve statistical significance (a further 8 years); and (iii) we believe it plausible that DSC cabinets, being a permanent wall fixture, may be more likely to accumulate spores (unlike the RSC which are removed and decontaminated monthly on average). We were satisfied that DSC most likely had a higher carriage rate, or at least, did not have a lower carriage rate and Pogorzelska-Maziarz's findings could not be causally-related to DSC/RSC differences. Notwithstanding which container type may have a higher *C. difficile* carriage rate, we believe it is not scientifically plausible for either to play a role in CDI transmission.

Strength of Evidence of RSC association with *C. difficile* Transmission

There are 8 criteria for evaluating the strength of evidence for environmental sources of infection:[33] (i) the organism can survive on the fomite; (ii) be cultured from the fomite; (iii) can proliferate on the fomite; (iv) acquisition of infection cannot be explained by other modes of transmission; (v) retrospective case-control studies show an association between exposure to the fomite and infection; (vi) prospective case-control studies may be possible when more than one fomite is in use; (vii) prospective studies in a subset of patients show an association between exposure and infection; (viii) decontamination of the fomite results in elimination of infection transmission.

In CDI transmission, 1 of the 8 criteria is irrelevant (*C. difficile* spores do not need to proliferate on the fomite); however our results and epidemiological literature show that 5 of the remaining 7 criteria cannot be met by sharps containers.

Endeavors to investigate reservoirs and additional routes of transmission of *C. difficile* are laudable, but they must have scientific feasibility. We estimate that since their inception in 1986, approximately 100 million RSC have been used worldwide, however, in this time, with the exception of the Pogorzelska-Maziarz paper[19] and the two papers mentioned above,[14, 15] in the many patient-room "surface-touch" studies in the world literature,[10-12,16,28,31,37,39] none have mentioned

sharps containers as a touched item during observational studies nor have sharps containers been mentioned as a potential fomite in U.S. *C. difficile* Guidelines.[6,17,18] We acknowledge "absence of evidence is not evidence of absence",[46] however in the decades of intensive searching for CDI fomites, sharps containers have not been implicated as a fomite – we believe on sound epidemiological and microbiological grounds.

Our findings are in agreement with a "probability of occurrence" paper that found the probability of disease-transmission risk with reusable waste bins was less than 1 in 400 million.[47]

We believe the Pogorzelska-Maziarz methodology to be sound but a scientific explanation for the DSC association was not proposed. The association of any sharps container with CDI is at odds with Chain of Infection principles,[29,30] CDI transmission studies,[10-12,16,28,31,37,39] CDI guidelines,[6,17,18] the clinical practices taught at our facilities, and guidelines surrounding sharps container and glove usage.[6,17,20,44,45] In examining epidemiological, microbiological, Chain of infection and Tests of Evidence criteria, we could find no scientific evidence or mechanism whereby sharps containers could be implicated in CDI transmission. We believe the statistically significant association of DSC with lower CDI found by Pogorzelska-Maziarz is not a causal relationship but is a casual correlation as may be found in unrelated events.[48]

Limitations: The number of containers sampled in CDI patient rooms was sub-optimal (our statistical power calculation indicated impractical sample-numbers) however, the difference found (16% vs 8%) allowed us to state, with 90% confidence, that DSC cabinets had a higher *C. difficile* carriage rate. Microbiological sampling was not conducted on RSC on arrival at the healthcare facility as our results indicate RSC arrive at healthcare facilities spore-free (see Discussion). The enrolment of study hospitals was not random however we believe their clinical practices with regard to sharps container use and glove use are compliant with national guidelines and are unlikely to be dissimilar from other hospitals. The study hospitals enrolled and the microbiological findings in these clinical settings may not be representative of all U.S. hospitals nor of all RSC commercially available in US. Recording the length of time containers had been in the room prior to the CDI patient was impractical and considered of minor importance as all DSC were housed in permanent cabinets and RSC were replaced monthly on average. The personnel swabbing containers in patient-rooms were not blinded as to the purpose of the swabbing, however all were either Infection Control and Prevention practitioners or Infectious Disease physicians trained in the technique. Finally, this investigation did not include real-time observation of processes of care for CDI patients to document staff movement after disposal of sharps.

Strengths: Collaborative authors' knowledge and clinical experience; Multi-facet study encompassing (i) extensive review of epidemiological literature, (ii) microbiological sampling of full RSC, (iii) microbiological confirmation that RSC decontamination process removed very high *C. difficile* challenges, (iv) comparative microbiological sampling of RSC and DSC in CDI patient rooms. Additional strengths were the random, large, geographically widespread RSC sampling in Stage 1; large area of sampling on containers; having access to a *C. difficile* reference laboratory; two links being broken in the Chain of Infection; and the non-fulfilment of 5 of 7 tests-of-evidence for sharps containers having a role in CDI transmission.

Conclusions

We conclude that: DSC and RSC have a low density and low frequency of *C. difficile* carriage; carriage is not higher in RSC; *C. difficile* bioburden on sharps containers in CDI patient rooms is below that required for an infective *C. difficile* dose; *C. difficile* spores on RSC are completely removed in the RSC factory decontamination process; and that neither DSC nor RSC play a role in *C. difficile* transmission to patients or staff. In applying the above epidemiological and microbiological results, the Chain of Transmission, and Strength of Evidence criteria, we conclude that, although a statistical association was found by Pogorzelska-Maziarz, the assertion that sharps containers may be a fomite for *C. difficile* transmission is not scientifically feasible.

References

1. Lessa F, Mu Y, Bamberg W, Beldavs Z, Dumyati G, Dunn J, et al. Burden of Clostridium difficile Infection in the United States. N Engl J Med 2015;372:825-34. doi: 10.1056/NEJMoa1408913.
2. National and State Healthcare-Associated Infections Progress Report (2014 data). National Healthcare Safety Network (NHSN), Centers for Disease Control and Prevention, Atlanta GA. Mar 2016. https://www.cdc.gov/HAI/pdfs/progress-report/hai-progress-report.pdf. Accessed April 24, 2017.
3. Magill S, Edwards J, Bamberg W, Beldavs, Z, Dumyati G, Kainer M, et al. Multistate Point-Prevalence Survey of HealthCare-Associated Infections. N Engl J Med 2014;370(13):1198-208. doi: 10.1056/NEJMoa1306801.
4. Weber D, Rutala W, Miller MB, Huslage K, Sickbert-Bennett E. Role of surfaces in the transmission of emerging health care associated pathogens: Norovirus, Clostridium difficile, and Acinetobacter species. Am J Infect Control 2010;38:S25-33. doi:10.1016/j.ajic.2010.04.196.
5. Weber DJ, Rutala WA. The Role of the Environment in Transmission of *Clostridium difficile* Infection in Healthcare Facilities. Infect Control Hosp Epidemiol 2011(3): 207-209. doi: 10.1086/658670.
6. McDonald LC, Gerding DN, Stuart 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 Inf Dis 2018;66:e1-48. DOI: 10.1093/cid/cix1085
7. 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
8. Starr JM, Campbell A, Renshaw E, Poxton IR, Gibson GJ. Spatio-temporal stochastic modelling of *Clostridium difficile*. J Hosp Infect **2009**; 71:49–56.
9. Eyre DW, Griffiths D, Vaughan A, et al. Asymptomatic *Clostridium difficile* colonization and onward transmission. PLoS One **2013**; 8:e78445.
10. Samore MH, Ventkataraman L, DeGirolami PC, Arbeit RD, Karchner AW. Clinical and molecular Epidemiology of Sporadic and Clustered Cases of Nosocomial Clostridium difficile Diarrhea. Am J Med 1996;100:32-40.
11. Best EL, Fawley WN, Parnell P, Wilcox MH. The Potential for Airborne Dispersal of Clostridium difficile from Symptomatic Patients. Clin Infect Dis 2010; 50(11):1450–1457. doi: 10.1086/652648.
12. Huslage K, Rutala WA, Sickbert-Bennett E, Weber DJ. A Quantitative Approach to Defining "High-Touch" Surfaces in Hospitals. *Infect Control Hosp Epidemiol* 2010; 31(8):850-853. doi: 10.1086/655016.
13. Guerrero DM, Nerandzic MM, Jury LA, Jinno S, Chang S, Donskey CJ. Acquisition of spores on gloved hands after contact with the skin of patients with Clostridium difficile infection and with environmental surfaces in their rooms. Am J Infect Control 2012;40:556-8. doi:10.1016/j.ajic.2011.08.002.
14. Neely AN, Maley MP, Taylor GL. Investigation of single-use versus reusable infectious waste containers as potential sources of microbial contamination. Am J Infect Control 2003;31:12-7. doi:10.1067/mic.2003.17
15. Runner J. Bacterial and viral contamination of reusable sharps containers in a community hospital setting. Am J Infect Control 2007;35:527-30. doi:10.1016/j.ajic.2006.09.017.
16. Sjoberg M, Eriksson M, Andersson J, Noren T. Transmission of Clostridium difficile spores in isolation room environments and through hospital beds. ActaPathMicroImmScan 2014;122:800–803.
17. APIC Guide to Preventing Clostridium difficile Infections 2013. APIC Implementation Guide. Association for Professionals in Infection Control and Epidemiology. http://apic.org/Resource_/EliminationGuideForm/59397fc6-3f90-43d1-9325-e8be75d86888/File/2013CDiffFinal.pdf Accessed May 9, 2017.
18. Dubberke ER, Carling P, Carrico R, Donskey CJ, Loo VG, L. McDonald C, Maragakis LL, Sandora TJ, Weber DJ, Deborah S. Yokoe DS, Gerding DN. SHEA-IDSA Practice Recommendation. Strategies to Prevent Clostridium difficile Infections in Acute Care Hospitals: 2014 Update. Infect Control Hosp Epidemiol 2014;35(6):628-645. doi: 10.1086/676023.
19. Pogorzelska-Maziarz, M. Relationship between sharps disposal containers and Clostridium difficile infections in acute care hospitals. Amer J Infect Contr 2015;43:1081-5. doi.org/10.1016/j.ajic.2015.06.007.
20. Dikon A. A response to the relationship between different types of sharps containers and Clostridium difficile infection rates in acute care hospitals. Am J of Infect Control 44 (2016) 612-8. doi.org/10.1016/j.ajic.2015.09.036.
21. Pogorzelska-Maziarz, M. Reply to Dikon in response to "A response to the relationship between different types of sharps containers and *Clostridium difficile* infection rates in acute care hospitals". Amer J Infect Contr 2016;14:855-6. doi.org/10.1016/j.ajic.2015.12.023.
22. Cadnum J, Hurless K, Deshpande A, Nerandzic M, Kundrapu S, Donskey C. Sensitive and Selective Culture Medium for Detection of Environmental *Clostridium difficile* Isolates without Requirement for Anaerobic Culture Conditions. J Clin Microbiol 2014;52(9):3259. DOI:10.1128/JCM.00793-14.
23. Kane S. Sample Size Calculator. ClinCalc: http://clincalc.com/stats/SampleSize.aspx. Updated July 1, 2017. Accessed March 13, 2018.
24. Wilcox MH, Bennett A, Best EL, Fawley WN, Parnell P. Reply to Snelling et al. Clin Infect Dis 2010;51:1104-1105.
25. Donskey CJ. Preventing Transmission of Clostridium difficile: Is the Answer Blowing in the Wind? Clin Infect Dis 2010; 50(11):1458–1461. DOI: 10.1086/652649.

26. Nerandzic M, Donskey C. Effective and Reduced-Cost Modified Selective Medium for Isolation of *Clostridium difficile*. J Clin Microbiol 2009;47(2):397-400. doi:10.1128/JCM.01591-08.
27. Meschke S, Smith BD, Yost M, et al. The effect of surface charge, negative and bipolar ionization on the deposition of airborne bacteria. J Appl Microb 2009;106:1133-1139. doi:10.1111/j.1365-2672.2008.04078.
28. Kim K, Fekety R, Batts D, Brown M, Silva J, Waters D. Isolation of Clostridium difficile from the Environment and Contacts of Patients with Antibiotic-Associated Colitis. J Infect Dis 1981;143(1):42-50.
29. Principles of Epidemiology in Public Health Practice. 3rd Ed. An Introduction to Applied Epidemiology and Biostatistics, Oct 2006 (Updated May 2012). U.S. Dept of health & Human Services, Centers for Disease Control and Prevention (CDC), Office of Workforce and Career Development, Atlanta, GA. https://www.cdc.gov/ophss/csels/dsepd/ss1978/ss1978.pdf Accessed June 13, 2017.
30. Break the Chain of Infection. Association for Professionals in Infection Control and Prevention, Arlington, VA, USA. 2016. http://professionals.site.apic.org/10-ways-to-protect-patients/break-the-chain-of-infection/ Accessed May 4, 2017.
31. Fekety R, Kim K-H, Brown D, Batts DH, Cudmore M, Silva J. Epidemiology of Antibiotic colitis: Isolation of Clostridium difficile from the hospital. Am J Med 1981;70:906-8.
32. Leggett HC, Cornwallis CK, West SA (2012) Mechanisms of Pathogenesis, Infective Dose and Virulence in Human Parasites. PLoS Pathog 8(2): e1002512. doi:10.1371/journal.ppat.1002512.
33. Guidelines for Environmental Infection Control in Health-Care Facilities. Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC), 2003. U.S. Dept HHS, (CDC) Atlanta, GA. https://www.cdc.gov/infectioncontrol/pdf/guidelines/environmental-guidelines.pdf Accessed May 4, 2017.
34. Goldstein EJC, Johnson S, Maziade P-J, McFarland LV, Trick, W, et al. Pathway to Prevention of Nosocomial Clostridium difficile Infection. Clin Infect Dis 2015;60(S2):S148–58. doi: 10.1093/cid/civ142.
35. Ali S, Muzslay M, Wilson P. 2015. A novel quantitative sampling technique for detection and monitoring of Clostridium difficile contamination in the clinical environment. J Clin Microbiol 53:2570 – 2574. doi:10.1128/JCM.00376-15.
36. Landelle C, Verachten M, Legrand P, Girou E, Barbut F, Brun-Buisson C. 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. doi: 10.1086/674396.
37. Adams CE, Smith J, Watson V, Robertson C, Dancer SJ. Examining the association between surface bioburden and frequently touched sites in intensive care. J Hosp Infect 2017;95:76-80. doi.org/10.1016/j.jhin.2016.11.002.
38. Best E, Parnell P, Thirkell G, Verity P, Copland M, Else P, et al. Effectiveness of deep cleaning followed by hydrogen peroxide decontamination during high Clostridium difficile infection incidence. J Hosp Infect 2014;87:25-33. doi.org/10.1016/j.jhin.2014.02.005.
39. Dancer S. Controlling Hospital-Acquired Infection: Focus on the Role of the Environment and New Technologies for Decontamination. Clin Micro Rev 2014;27(4):665-90. doi:10.1128/CMR.00020-14
40. Maurer IM. Ch5 Cleaning the hospital: Care with water. In: Hospital Hygiene. 3rd Ed, p52. Richard Clay (The Chaucer Press) Ltd, Suffolk UK; 1985.
41. Lawley T, Clare S, Deakin L, Goulding D, Yen J, Raisen C, et al. Use of Purified Clostridium difficile Spores to Facilitate Evaluation of Health Care Disinfection Regimens. App Envir Micro 2010;76(20):6895-900. doi:10.1128/AEM.00718-10.
42. Kanwar A, Mana TSC, Cadnum J, Alhmidi H, Koganti S, Donskey CJ. How well does transfer of bacterial pathogens by culture swabs correlate with transfer by hands? Am J Infect Control 2017 (in press). doi.org/10.1016/j.ajic.2017.03.001.
43. 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 of Infect Contr 2017; 45:51-8. doi.org/10.1016/j.ajic.2016.08.017.
44. APIC Guide to Hand Hygiene Programs for Infection Prevention. APIC Implementation Guides, Association for Professionals in Infection Control and Epidemiology. https://apic.org/Resource_/EliminationGuideForm/9fae54e1-ebb9-44f9-a5b2-1079565d6c34/File/To%20download%20the%20PDF%20of%20Guide%20to%20Hand%20Hygiene%20Programs%20for%20Infection%20Prevention.pdf. Accessed March 26, 2017.
45. Occupational Safety and Health Administration. Toxic and hazardous substances. Standard number 1910.1030. Bloodborne pathogens. www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051. Accessed March 26, 2018.
46. Altman DG, Bland JM. Absence of evidence is not evidence of absence. BMJ 1995;311:485.
47. Grimmond T. Using reusable containers for hospital waste – is there an infection risk? South Afr J Epidemiol Infect 2013;28(4):197-201.
48. Matthews R. Storks Deliver Babies (p=0.008). Teaching Statistics. Volume 22, Number 2, Summer 2000. http://robertmatthews.org/wp-content/uploads/2016/03/RM-storks-paper.pdf Accessed June 13, 2017.