PEER-REVIEWED ARTICLE

Food Protection Trends, Vol 40, No. 6, p. 392–401

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Characterizing Microbial Cross-Contamination on Large Surfaces Using a Traditional "Cloth and Bucket" Disinfection Method

ABSTRACT

Use of buckets containing soiled disinfectant solutions for disinfection is regularly practiced in food service and other settings. This study characterized microbial transfer of vegetative bacteria (*Listeria innocua* and *Escherichia coli*), spores (*Bacillus cereus*), and a virus (MS2 bacteriophage), to large surfaces, using a "cloth and bucket" method with a commonly used quaternary-ammonium compound (QAC) disinfectant (with or without 5% soil) and a phosphate-buffered-saline (PBS) control. We also characterized concentrations of organisms in the bucket solutions after wiping. With disinfectant (with or without soil), there was little transfer of vegetative bacteria. Transfer occurred readily with the PBS control (4.8 ± 1.0 and 3.3 ± 0.9 log CFU/surface for *Listeria innocua* and *Escherichia coli*, respectively). Spores were transferred efficiently, regardless of whether PBS or QAC was used or whether test was with or without soil (range, 6.5 to 7.8 log CFU/surface). MS2 bacteriophage appeared to be eliminated relatively quickly. When the QAC did not inactivate the organism (regardless of soil load), high microbial loads (≥ 87.9% of initial inoculum) were detected in the bucket solution after wiping experiments. This study suggests that reusable cloth can potentially promote contamination of surfaces, sometimes in the presence of disinfectant. This is concerning for food service and other settings in which disinfection practices rely on the cloth and bucket system.

INTRODUCTION

Continuous use of buckets containing soiled water or disinfectant solutions for disinfection of surfaces is a regular practice in food service establishments, schools, and many other settings. It is not uncommon to use a single sponge or cloth multiple times over a day or a shift to clean environmental surfaces. However, little is known about the safety of this practice, except that, microbiologically speaking, it would be expected to be hazardous because of concerns about consistent risk for cross-contamination.

There have been many studies to characterize the transfer of microbes among surfaces, hands, and foods but very few attempting to quantify this phenomenon as a function of

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cloth use or wiping and even fewer looking at viral pathogens. Smith et al. *(16)* observed that, when using clean, wetted wipes applied to surfaces previously inoculated with 10^5 CFU of common nosocomial bacteria (specifically methicillin-resistant *Staphylococcus aureus*, spores of *Clostridium difficile*, and *Escherichia coli*), between 2 and 3 log CFU of the initial inoculum was removed, showing some mechanical removal of organisms by the action of wiping. Bergen et al. *(4)* observed cross-contamination to microfiber cloths from surfaces inoculated with 10^4 CFU *Enterococcus faecalis* and spores of *Bacillus cereus*. However, Rossi et al. *(12)* observed cross-contamination of 0.01 to 1% of initial bacterial load to clean surfaces upon wiping with naturally contaminated industrial sponges. Gibson et al. *(8)* demonstrated that the efficiency of transfer of several viruses (specifically murine norovirus, feline calicivirus, GI.1 human norovirus [HNV], and bacteriophages PRD1 and MS2) to acrylic and stainless steel surfaces was dependent upon both the virus and the cloth type.

A recurring difficulty with trying to quantify the degree of cross-contamination associated with wiping events is standardization of experimental protocols because key parameters (e.g., pressure, distribution of force, and the mechanics of the wiping motion) can have a significant effect on results. A machine called the Wiperator (Filtaflex, Almonte, Canada) simulates the orbital action of wiping and allows presetting of pressure, duration, and the number of wiping strokes. Although there is a standardized method for the Wiperator *(2)*, only two published articles have documented its use, one focused on a multilaboratory validation of instrument performance using sanitizing wipes *(13)*, and the other investigated the efficacy of various detergent wipes to remove and transfer common nosocomial bacterial pathogens from stainless steel surfaces *(11)*; in both, only wiping on very small surfaces was characterized. To our knowledge, there have been no systematic studies describing the degree to which cross-contamination occurs when using soiled cloths to disinfect surfaces in a real-world, scaled-up setting.

The aim of this study was to characterize the degree of cross-contamination of representative gram positive and negative bacteria (specifically, *Listeria innocua* and *E. coli*), spores (*Bacillus cereus*), and viruses (MS2 bacteriophage as a human enteric virus surrogate), to large surfaces, with a traditional "cloth and bucket" method and a commercial quaternary ammonium compound (QAC) disinfectant commonly used in restaurant settings (with and without additional soil) and a phosphate-buffered saline (PBS) (no disinfectant) control. In addition, we characterized the concentrations of these organisms transferred to, and remaining in, the bucket disinfectant solutions after wiping with the cloth and bucket method.

MATERIALS AND METHODS

Bacterial cultures and enumeration

Listeria innocua (ATCC 33091) and *Escherichia coli* (ATTC 25922) were selected for this study as surrogates for *Listeria monocytogenes* and pathogenic *E. coli*, respectively. Overnight cultures of *L. innocua* and *E. coli* were prepared in 10 mL of tryptic soy broth, with shaking at 140 rpm at 37°C for 21 to 27 h. Cultures were then centrifuged (model 5810R, Eppendorf, Hamburg, Germany) at room temperature at 2,200 relative centrifugal field (RCF) for 15 min. Pellets were recovered and resuspended in 10 mL of PBS and centrifuged again at room temperature at 2,200 RCF for 15 min. Pellets were then recovered and resuspended in a final volume of 600 µL of PBS, for a final concentration of 10^8 to 10^9 CFU/mL. Immediately before inoculating surface 1–dirty (S1d), the cultures were combined for a final volume of approximately 1.2 mL. Enumeration of bacteria after swabbing was done by plating serial dilutions in PBS on modified oxford agar or MacConkey Agar at 37°C overnight (18 to 20 h) for *L. innocua* and *E. coli*, respectively.

Bacteriophage culture and enumeration

MS2 coliphage (ATCC 15597-B1) stocks at a concentration of 10^9 to 10^{10} PFU/mL were used as initial inoculum. Stock solutions were prepared per the protocol described in U.S. National Science Foundation standard 55 *(3)*. Enumeration of MS2 was performed on serial dilutions by the double agar layer method in accordance with the method of Su and D'Souza *(17)* with *E. coli* F+ C3000 cells as host (ATCC B-15597).

Spore culture and enumeration

Bacillus cereus spores (ATCC 49063) at a concentration of approximately 10^8 to 10^9 CFU/mL were produced according to Johnson et al. *(9)*. Spores were harvested in sterile distilled water, held at 4°C for 72 h to ensure lysis of vegetative cells, and stored in glycerol at 4°C until use. Absence of vegetative cells in the stock solution was confirmed by phase-contrast microscopy. Enumeration of spores was done by plating serial dilutions on tryptic soy agar and followed by incubation at 37°C overnight.

Surface inoculation and wiping experiments

Laminate countertops were purchased from Home Depot (catalog 1000018831, Atlanta, GA) and sectioned into multiple 2-ft (0.6 m) by 3-ft (0.9 m) (6 ft^2 [0.56 m^2] surfaces. A diagram of the entire workspace is shown in *Fig. 1a* and *Fig. 1b*. One hour before inoculation, all surfaces were disinfected by spraying with 10% bleach (5-min contact time) and wiping with a clean, disposable paper cloth. This was followed by spraying with 70% ethanol and wiping with a clean, disposable paper cloth. The surfaces were then allowed to naturally air dry for 1 h. A cleaning validation or negative control sample was taken by swabbing a 12-in (30.5 cm) by 12-in (1 ft^2 [0.09 m^2]) area of S1d *(Fig. 1b)* with a sampling template (catalog 900206, Environmental Monitoring Systems, North Charleston, SC) with an environmental sampling swab in 10 mL D/E neutralizing broth (catalog

FIGURE A

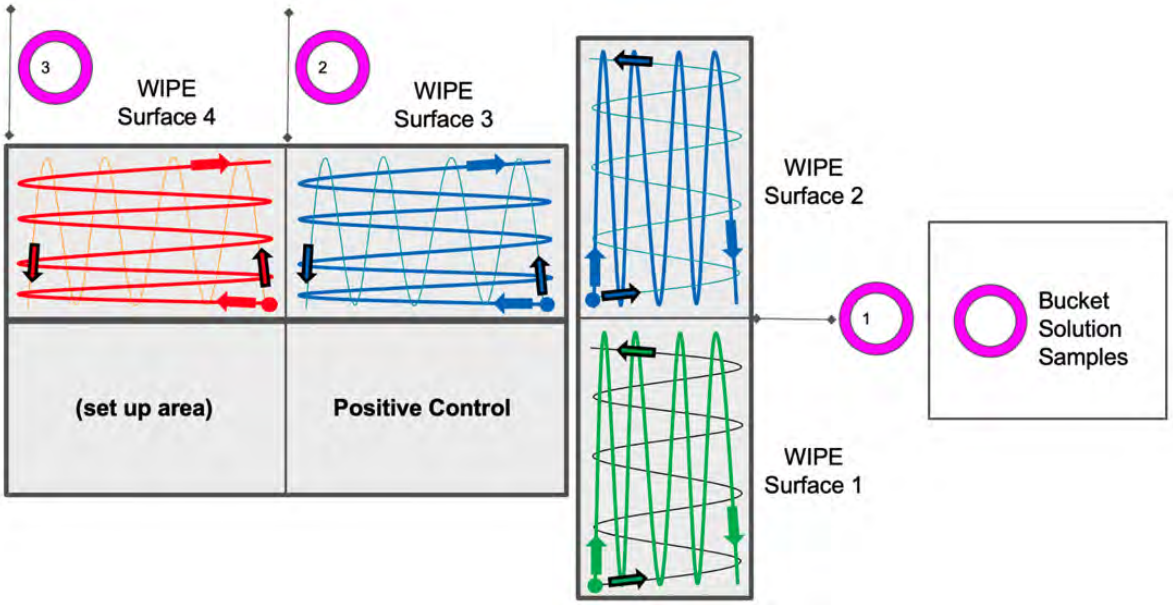

FIGURE B

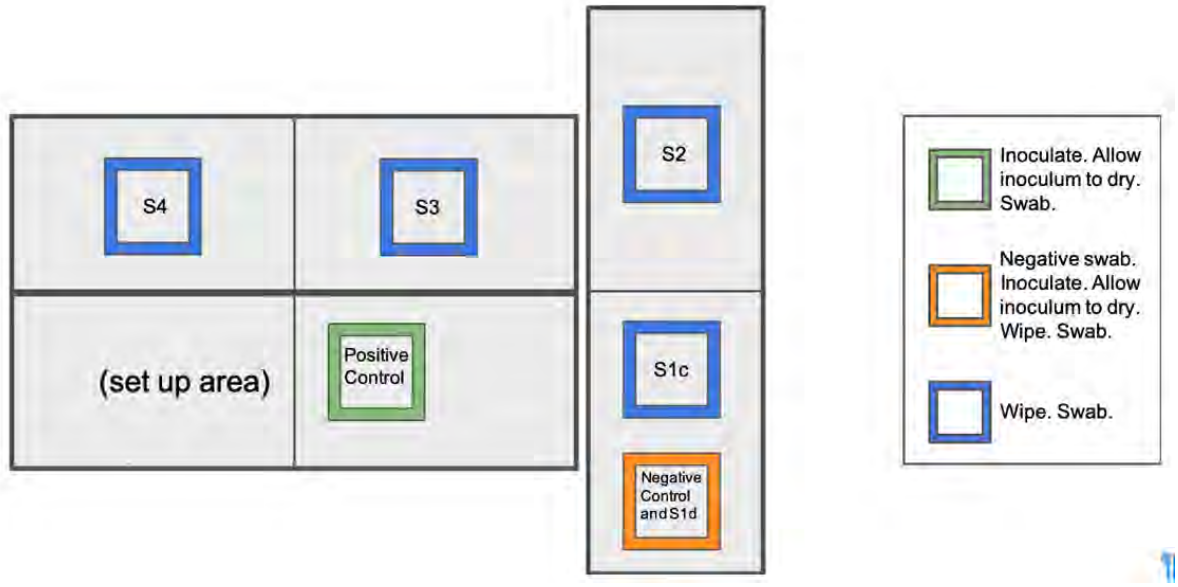

FIGURE 1. Overall experimental design. (a) A diagram of the wiping protocol, including the timing for collection of water samples drawn from the wiping bucket solution. (b) Diagram of the swabbing locations for each of the individual laminate surfaces.

EZ-10HC-PUR, EZ Reach Sponge, World Bioproducts, Libertyville, IL, with a 1.5- by 3-in. [3.8- by 7.6-cm] sponge) before inoculating the surface. The swabbing procedure was as follows, with swabbing locations shown in *Fig. 1b*. First, as much of the broth as possible was squeezed out of the swab. Starting at the top left, swabs were swiped within the template area from left to right, right to left, ending at the bottom right (as many swipes as needed). The swab was flipped over and the surface was reswabbed in the same manner starting at the bottom right, from bottom to top, top to bottom, ending in the top left (as many swipes as needed). This was repeated two more times in both diagonal directions. The handle of the swab was twisted off, and the swab was returned to the bag containing the neutralizing broth.

After the negative-control surface was swabbed (*Fig. 1b*; Neg S1d), the positive-control surface and S1d *(Fig. 1b)* were inoculated with one of the microbial suspensions (prepared as described above). For both the positive control surface and the S1d *(Fig. 1b)*, a 12- by 12-in (1 ft^2) inoculation area was designated, and 25 spots of 20 µL each of the inoculum (500 µL total inoculum volume) was placed over each 1-ft^2 area. The inoculum was allowed to dry before wiping experiments took place. Drying of the inoculum ranged from 45 min to 2 h, was dependent on the surrogate, and was confirmed by visual inspection. The entire 1-ft^2 inoculated area of the positive control surface was swabbed as described above.

The surface wiping procedure is diagrammed in *Fig. 1a*. A standard terry cloth bar rag (catalog B00KKRCS2Q, All In Safety, Bloomfield, NJ), 16 in. (40.6 cm) by 19 in. (48.3 cm), was folded in half and then into thirds, resulting in a 50-in2 (322.6-cm^2) wiping area. The folded towel was placed into a bucket containing one of three solutions: (i) PBS (2 L), used as a no disinfectant control; (ii) Oasis 146 (Ecolab, Saint Paul, MN), prepared per manufacturer's instructions (QAC; 2 L hard water + 7.8 mL concentrated disinfectant; final target disinfectant concentration of 400 ppm), used as a representative "clean" disinfection solution; or (iii) QAC prepared per manufacturer's instructions (described in *(2)*), with an additional 5% soil load prepared according to an ASTM standard *(1)*, used as a representative "dirty" disinfection solution. The cloth was submerged in the bucket solution and used to manually mix the bucket's contents with a gloved hand by swirling the contents in a circular motion. After thorough wetting, the cloth was squeezed out by hand and was then used to wipe in a back-and-forth motion from S1d to surface 1–clean (S1c). Wiping started at the top left corner of S1d (where the dried inoculum was located), and the cloth was used to wipe top left to top right, right to left, left to right, ending in the bottom right corner of S1c with a total of eight swipes. Wiping was then repeated starting back in the top left to bottom left, bottom to top, top to bottom, ending in the top right with a total of eight swipes.

The folded cloth was then placed back into the bucket of solution, squeezed out, and used to wipe surface 2 (S2) in the same back and forth motion described above *(Fig. 1a)*, making sure to use the same area of the cloth surface for wiping. The cloth was then immediately used to wipe surface 3 (S3; *Fig. 1a*), before being placed back into the bucket with solution and squeezed out before wiping surface 4 (S4; *Fig. 1a*) in the same back and forth motion described above.

A 1-ft^2 swab sample was taken from all surfaces *(Fig. 1b)* using the swabbing procedure described above for the negative control surface. Microorganisms were eluted from the swabs by squeezing the swab in the neutralizing buffer 120 times between two fingers before enumeration by plating serial dilutions of the neutralizing broth in PBS on selective medium with incubation as described above in enumeration methods for each microorganism. Microorganism counts from the 1-ft2 sampling areas were adjusted to reflect number of microorganisms present on the entire 6-ft^2 surface.

Bucket solution sampling

Bucket solution was sampled immediately after wiping S1, S3, and S4 by drawing 1mL from the bucket using a pipette *(Fig. 1a)*. The sample was transferred to a 15-mL conical tube containing 9 mL of D/E neutralizing broth. Samples were processed for enumeration of microorganisms as described above. In all cases, no difference was seen in the counts from the three 1-mL solution samples (data not shown). Results were calculated to reflect the total number of organisms remaining in the entire 2-L volume of the bucket solution by averaging the counts from the three 1-mL solution samples and multiplying by 2,000.

Statistical analysis

All experiments were independently replicated in triplicate on separate days. Results are presented as mean total CFU or PFU per 6 ft^2 surface ± standard deviation and as a percentage of the total CFU or PFU transferred to, and remaining in, the bucket solution for liquids (Excel, Microsoft Corporation, Redmond, WA).

RESULTS AND DISCUSSION

In food service establishments, schools, and many other settings, the use of the cloth and bucket method for cleaning environmental surfaces is common. Because little is known about the potential for these practices to spread pathogenic bacteria, viruses, and spores, the aim of this study was to characterize the spread of representative gram-positive and gram-negative vegetative bacteria, a spore, and a surrogate virus from laminate surface to laminate surface using a scaled-up "real life" experimental design. In addition, we sought to characterize the concentrations of those organisms transferred to, and remaining in, the bucket solutions after wiping events had been carried out because residual organisms could be the source of potential ongoing recontamination in real-world settings.

To replicate the initial contamination source, S1d was inoculated with the test microorganism; after which, a

TABLE 1. Cross-contamination efficiency ratios of microorganisms from an inoculated laminate surface to a clean laminate surface with a single wiping step using the cloth and bucket method

Organism	Treatment	CFU/PFU on surface 1–dirty (mean ± standard deviation)	CFU/PFU on surface 1–clean (mean ± standard deviation)	Cross-contamination efficiency (mean ± standard deviation)[a]
L. innocua	PBS	7.24 ± 0.99	6.79 ± 0.88	1.08 ± 0.06
	QAC	3.77 ± 0.27	LOE[b]	N/A[b]
	QAC + 5% soil	4.18 ± 0.29	3.51 ± 0.38	1.20 ± 0.05
E. coli	PBS	5.26 ± 1.26	5.08 ± 1.29	1.05 ± 0.05
	QAC	3.19 ± 0.42	LOE[b]	N/A[b]
	QAC + 5% soil	3.72 ± 0.30	3.01 ± 0.40	1.28 ± 0.17
B. cereus	PBS	8.85 ± 0.06	8.75 ± 0.08	1.01 ± 0.01
	QAC	9.04 ± 0.34	8.90 ± 0.22	1.01 ± 0.02
	QAC + 5% soil	9.13 ± 0.16	9.20 ± 0.07	0.99 ± 0.02
MS2	PBS	6.34 ± 0.96	5.80 ± 0.86	1.09 ± 0.03
	QAC	5.51 ± 0.94	4.41 ± 0.78	1.26 ± 0.17
	QAC + 5% soil	5.50 ± 0.88	4.76 ± 0.22	1.15 ± 0.18

[a]Cross-contamination efficiency was calculated as a ratio of the total number of organisms on the inoculated side of S1d to the total number of organisms on S1c after the first wiping event (S1d/S1c).

[b]Not applicable (N/A), when the organism was completely inactivated by the disinfectant (limit of enumeration [LOE] reached) and ratios could not be determined.

terry-cloth bar cloth was dunked in a solution and used to wipe the contaminated area in a back and forth motion to the clean side of the surface (S1c). By determining the number of microorganisms remaining on S1d, and the number that were moved to S1c by the cloth during wiping, we were able to determine the efficiency of cross-contamination from the initial contamination source to a clean surface, also known as the initial cross-contamination event *(Table 1)*. The efficiency of cross-contamination was calculated as a ratio of the number of organisms enumerated from S1d to the number of organisms enumerated from S1c. A result close to 1.0 indicated that close to an equal number of the test organisms were found on both the inoculated and the cross-contaminated sides of S1 after wiping and that the organism was easily moved from one side of the surface to the other. Cross-contamination efficiency ranged from 0.99 to 1.28, showing the test organisms were moved efficiently from the inoculated side of S1 (S1d) to the clean side of S1 (S1c). In some cases, this occurred even when the disinfectant was used, with or without soil. This shows that the initial wiping of a contaminated surface using the traditional cloth and bucket method easily spreads organisms to clean areas of the same surface.

The efficacy of disinfection was dependent upon microorganism, bucket solution type, and sequence of wiping actions. These data are provided in *Figs. 2* through *Figs. 5* as log PFU or CFU transferred to a surface for each of the sequential wiping actions. For *E. coli* and *L. innocua*, when the bucket contained PBS alone (control), cross-contamination occurred with each sequential wiping step, although its efficiency reduced with subsequent wiping events *(Figs. 2 and 3)*. By the fourth wiping event, about 5-log less CFU were deposited on the clean recipient surface. Further, cross-contamination appeared less efficient if first preceded by rinsing the cloth in the bucket solution (as was the case for S1 vs. S2 and S3 vs. S4) as compared to two sequential wipes without a cloth rinse (S2 versus S3).

When the bucket contained the QAC solution, with or without added soil, the disinfectant quickly inactivated both *L. innocua* and *E. coli* and effectively prevented cross-contamination, which was negligible after the first wipe (S1c) and below assay enumeration limits (< 2.78 log CFU/surface) for S2, S3, and S4.

Overall, these results were expected because it is readily accepted that quaternary ammonium compounds are effective in inactivating vegetative gram-positive and gram-negative bacteria *(6)*. These data are consistent with the study of Scott and Bloomfield *(15)*, in which two different types of cloths were evaluated for cleaning in a food-preparation

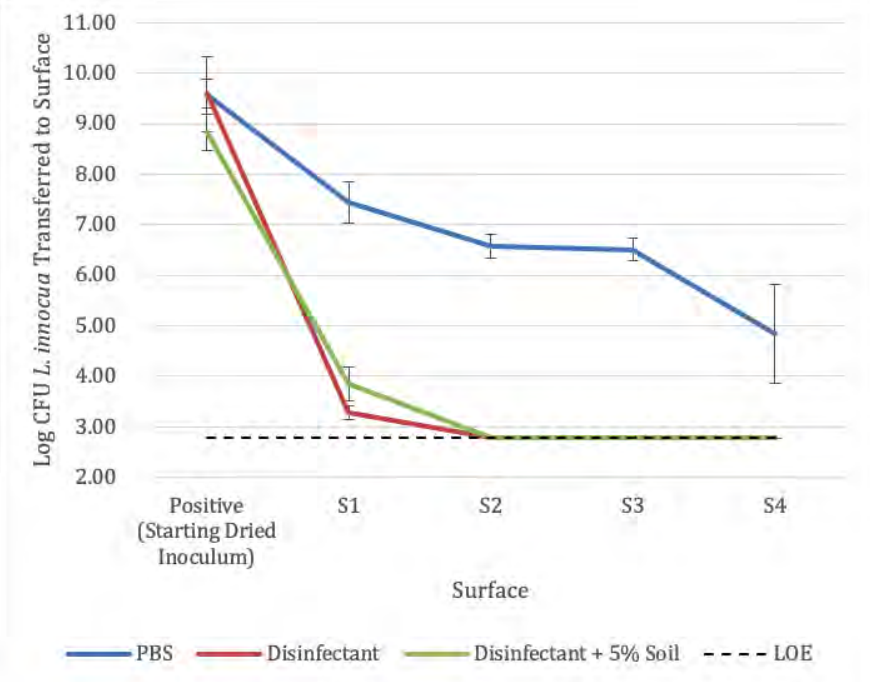

FIGURE 2. Log CFU of *Listeria innocua* transferred to, and remaining on, laminate surfaces during wiping experiments. Surface 1 was inoculated at the same level as the positive surface (starting dried inoculum) before wiping experiments. Cloth was used to wipe surfaces sequentially from surface 1 through surface 4 and was submerged in the bucket solution (PBS, disinfectant, or disinfectant + 5% soil) after wiping surfaces 1, 3, and 4, but not surface 2.

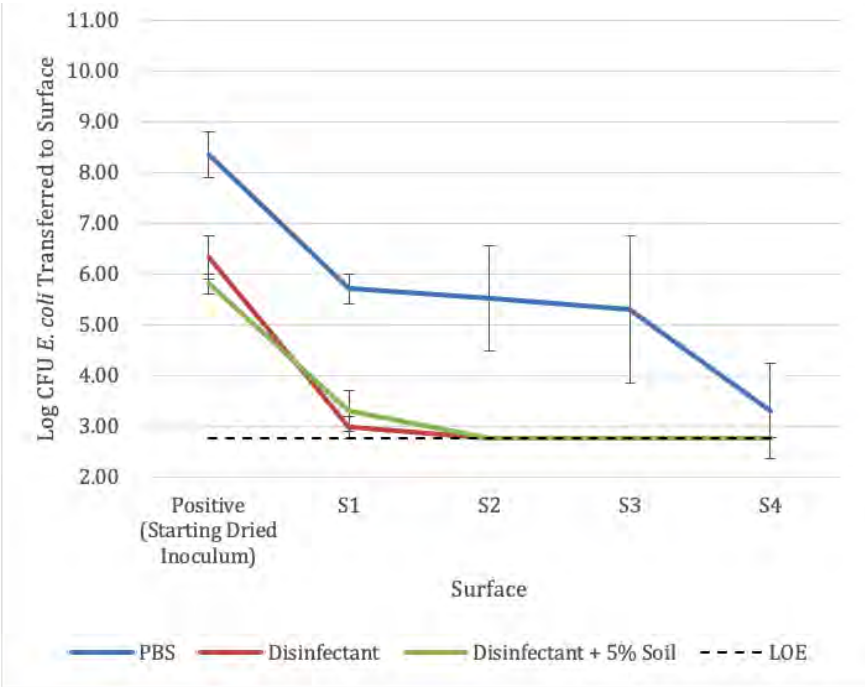

FIGURE 3. Log CFU of *Escherichia coli* transferred to, and remaining on, laminate surfaces during wiping experiments. Surface 1 was inoculated at the same level as the positive surface (starting dried inoculum) before wiping experiments. Cloth was used to wipe surfaces sequentially from surface 1 through surface 4 and was submerged in the bucket solution (PBS, disinfectant, or disinfectant + 5% soil) after wiping surfaces 1, 3, and 4, but not surface 2.

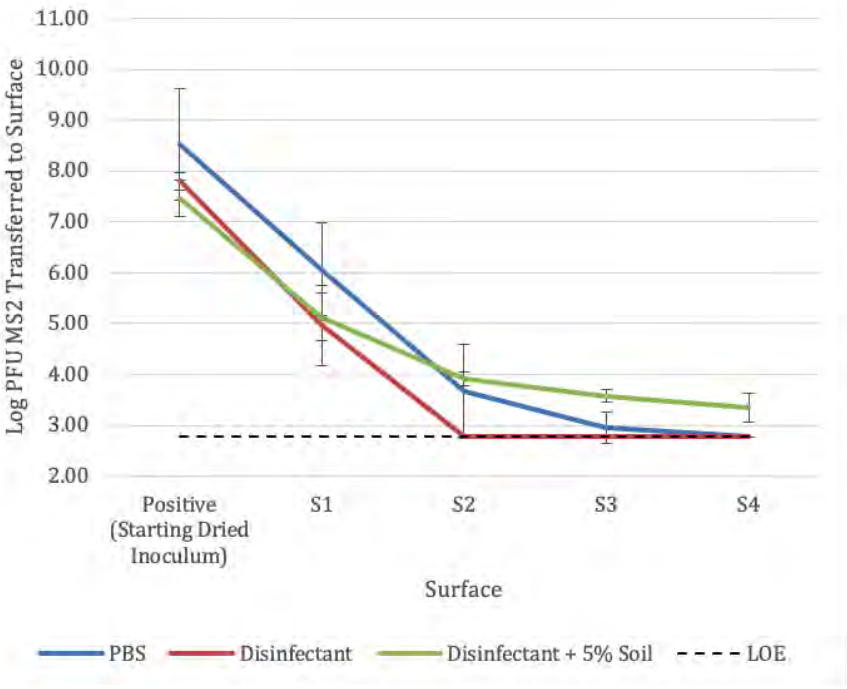

FIGURE 4. Log PFU of MS2 bacteriophage transferred to, and remaining on, laminate surfaces during wiping experiments. Surface 1 was inoculated at the same level as the positive surface (starting dried inoculum) before wiping experiments. Cloth was used to wipe surfaces sequentially from surface 1 through surface 4 and was submerged in the bucket solution (PBS, disinfectant, or disinfectant + 5% soil) after wiping surfaces 1, 3, and 4, but not surface 2.

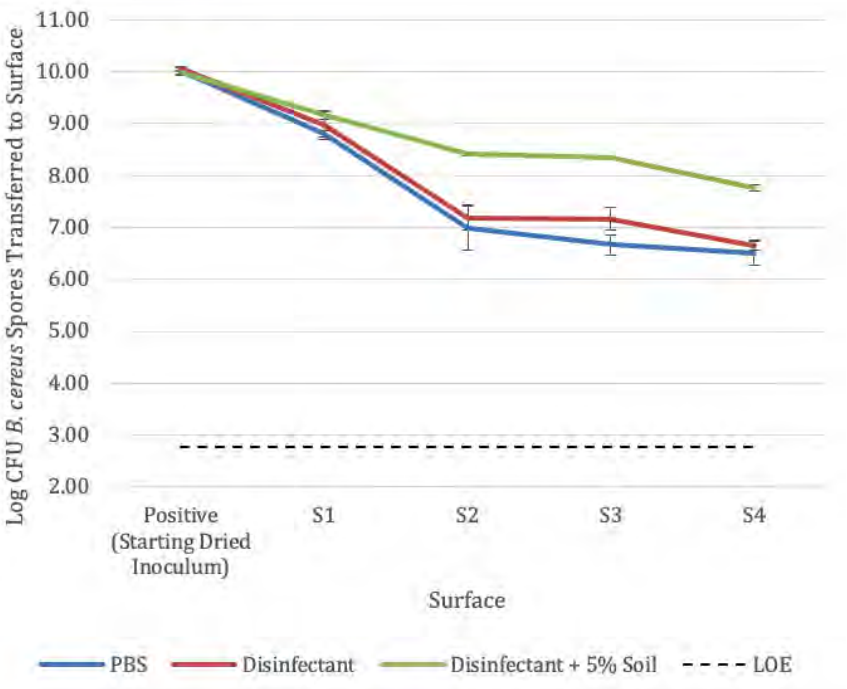

FIGURE 5. Log CFU of *Bacillus cereus* spores transferred to, and remaining on, laminate surfaces during wiping experiments. Surface 1 was inoculated at the same level as the positive surface (starting dried inoculum) before wiping experiments. Cloth was used to wipe surfaces sequentially from surface 1 through surface 4 and was submerged in the bucket solution (PBS, disinfectant, or disinfectant + 5% soil) after wiping surfaces 1, 3, and 4, but not surface 2.

area. They observed high aerobic plate counts for both cleaning cloths and on the associated surfaces after cleaning in the absence of a disinfectant. What was interesting in the current study was that the efficacy of the disinfectant was maintained even in the presence of a relatively high soil load, despite suggestions that a higher concentration of QAC is needed to be efficacious in the presence of high soil *(5)*. Medrano-Félix et al. *(10)* demonstrated that households in which a QAC disinfectant intervention was introduced had reduced numbers of *E. coli* on kitchen countertops over time, compared with households that did not receive the QAC intervention, which showed no change or increased numbers of *E. coli* on countertops. Our work also demonstrates the efficacy of QAC disinfectants against representative gram-negative and gram-positive bacteria, even in the presence of a significant organic load.

The results for bacteriophage MS2 are shown in *Fig. 4*. The results for the positive control (PBS as bucket solution, no disinfectant) showed that quantifiable cross-contamination occurred through wiping of S3. With the first wipe, approximately 2.5 log PFU/surface was removed and/or inactivated, giving a concentration of 6.1 ± 0.9 log PFU on S1. After submerging the cloth in PBS and using that cloth to wipe down S2, 3.7 ± 0.9 log PFU was transferred, with 3.0 ± 0.3 log PFU/surface then transferred to S3, representing subsequent cross-contamination. After submerging the cloth in PBS for a second time, the MS2 was not detected on S4 after the last wiping event, at least within the enumeration limit of the assay (which was < 2.78 log PFU/surface).

The data for QAC, with and without added soil, were similar but not identical to that for PBS. In the absence of added soil, MS2 became undetectable on S2, showing some benefit of the QAC in preventing long-term cross-contamination. For QAC with 5% soil experiments, cross-contamination was never completely ameliorated since quantifiable virus was present even on S4, albeit the concentrations of MS2 for S3 and S4 were low, at 3.6 ± 0.1 log PFU/surface and 3.4 ± 0.3 $\log_{10}$ PFU/surface, respectively. For all three treatments, rapid inactivation and/or removal of virus occurred between the positive control and S2; thereafter, inactivation or cross-contamination was either marginal or nonexistent (because the assay limit of enumeration had been reached).

MS2 was chosen for use as a surrogate for human enteric viruses, specifically HNV. It has been shown that HNV has long-term persistence (weeks) on surfaces, and in general, QACs have poor efficacy against HNV *(19)*. The results of this study were somewhat surprising if taken in the context of MS2 being used to model HNV behavior. The fact that so much of the virus was lost between the positive control and S2, whether or not the disinfectant was present, suggests that MS2 was effectively removed by the act of wiping. There is little information on the efficacy of rubbing to remove HNV, with one study showing approximately 1 log removal of HNV depending on the type of cloth used *(8)*. In the same study, the impact of cloth type on the transferability of HNV surrogates (MS2 and PRD1 bacteriophages and feline calicivirus) to stainless steel surfaces was evaluated and found to be cloth dependent, ranging from very little transferred to around 3 log PFU. Hence, we should be cautious in assuming that HNV would be removed by cloth wiping because there are many variables in our study that were not evaluated. Unfortunately, because removal appeared to be so effective, there was only a small window (about 1 log PFU) in which to evaluate efficacy of the QAC, not enough to make any compelling conclusions about sanitizer efficacy. In short, further studies are required to truly understand the behavior of HNV with respect to cross-contamination and inactivation in reusable cloth-and-bucket scenarios.

Results for wiping experiments with *B. cereus* differed quite considerably from those for the vegetative bacteria and MS2. In the absence of a disinfectant (PBS control), 8.8 ± 0.1 log CFU/surface remained on S1 after the first wiping step, suggesting that approximately 1.2 log CFU/surface was removed by the act of wiping when compared with the positive control. After submerging the cloth in PBS and then using that cloth to wipe down S2, a total of 7.0 ± 0.4 log CFU/surface was transferred. This degree of cross-contamination remained relatively consistent for subsequent wiping steps (S3 and S4). When QAC without added soil was used as the bucket solution, very similar results were observed, strongly suggesting that the QAC had no sporicidal effect on *B. cereus*. This was not unexpected because the efficacy of QACs on spores has been shown to be formulation dependent *(6)*. The addition of 5% soil to the disinfectant solution had something of a protective effect in wiping experiments, with only 0.8 log CFU/surface removed in the first wiping action and relatively consistent cross-contamination (8.4 ± 0.1 log CFU/surface, 8.3 ± 0.1 log CFU/surface, and 7.8 ± 0.1 log CFU/surface for S2, S3, and S4, respectively) occurring thereafter.

Collectively, *B. cereus* spores were readily transferred from surface to surface, regardless of the solution used for wiping experiments. The spores were resistant to inactivation by the QAC and may have been protected by the addition of soil. As was the case for the vegetative bacteria, cross-contamination appeared less efficient if first preceded by rinsing the cloth in the bucket water (as was the case for S1 versus S2 and S3 versus S4) as compared with two sequential wipes without a cloth rinse (S2 versus S3). These data clearly demonstrate the environmental resilience of spores and their ease of spread from surface to surface using reusable cloths, regardless of whether or not a QAC disinfectant is present. This was perhaps expected because some studies of disinfection of *C. difficile* spores have found that QAC-based disinfectants did not have sporicidal properties *(7)*. The results of this study support those of previous ones *(18)* showing that *C. difficile* spores are easily transferred by reusable cloths to surfaces across multiple wiping events.

TABLE 2. Numbers of microorganisms detected in bucket solution after wiping experiments

Organism	Treatment	Positive input control (mean CFU/PFU ± standard deviation)	Bucket solution (mean CFU/PFU ± standard deviation)	% transferred to and remaining in bucket solution[a]
L. innocua	PBS	9.58 ± 0.74	9.49 ± 0.99	99.1
	QAC	9.60 ± 0.28	N/A-LOE[b]	N/A-LOE[b]
	QAC + soil	8.83 ± 0.37	N/A-LOE[b]	N/A-LOE[b]
E. coli	PBS	8.13 ± 0.18	8.54 ± 0.78	105.0
	QAC	6.33 ± 0.43	N/A-LOE[b]	N/A-LOE[b]
	QAC + soil	5.81 ± 0.20	N/A-LOE[b]	N/A-LOE[b]
B. cereus	PBS	10.01 ± 0.07	10.54 ± 0.22	105.3
	QAC	10.06 ± 0.03	9.48 ± 0.19	94.2
	QAC + soil	9.98 ± 0.03	9.96 ± 0.03	99.8
MS2	PBS	8.52 ± 1.09	8.39 ± 0.99	98.5
	QAC	7.80 ± 0.18	N/A-LOE[b]	N/A-LOE[b]
	QAC + soil	7.46 ± 0.35	6.56 ± 0.38	87.9

[a]Transferred to, and remaining in, the bucket solution was calculated by dividing the number of organisms detected in the bucket solution by the number of organisms detected in the positive input control and multiplying by 100 [(bucket solution mean/positive input control mean) × 100].

[b]Not applicable (N/A), when the organism was completely inactivated by the disinfectant (limit of enumeration [LOE] reached) and ratios could not be determined.

It is important to note that the results shown in this study are limited to the organisms included, the use of selective media (which may prevent recovery of injured cells), presence of laminated surfaces, the QAC product chosen, and the use of terry-cloth bar towels. Because we did not account for injured cells, our results could have underestimated surviving populations on the surfaces and in the bucket water, meaning that the number of organisms transferred from one surface to another, and into the bucket solution, could have been higher. Future studies using nonselective medium or increasing the incubation time on selective medium would be an appropriate next step. In addition, it was not possible to standardize wiping pressure because of the large surface area of the surfaces studied. We also did not measure the concentration of the QAC after it was prepared (although we did follow manufacturer's instructions) because we were attempting to simulate a real-life scenario as much as possible. Front-of-house retail food service employees are not likely to measure QAC concentration in prepared solutions, and if they did, there is no record of the accuracy of those measurements. Although measuring the active ingredient concentration in studies such as these is not always common, measuring the QAC concentration, especially after the addition of soil, would have been interesting, but was outside the scope of this study. It has also been shown that the efficacy of QACs can be reduced by cloth towels *(5)*, and that effect was not characterized in the current study. Some common practices also include a cleaning step before disinfection, and a cleaning step was not characterized here. Hence, this study only evaluated disinfection, although it should be noted that the surfaces were very clean, with the exception of a very small amount of inoculum, at the onset of the experiments. Further method-development studies to optimize and standardize wiping actions, such as pressure on larger surfaces, are warranted, as discussed in Sattar and Maillard *(14)*, as well as those that investigated the effect of cleaning a surface before disinfection, and how QACs are affected by cloth towels in this scaled-up model.

The data for the microbial concentrations in bucket solutions after wiping are provided in *(Table 2)*. Note that, when organisms were present and not inactivated by the QAC, their concentrations were very high and represented >85% of the initial inoculum enumerated from the positive control surface. In all cases, high concentrations of the microbes were detected in the control (PBS) bucket solutions after wiping. This suggests that the absence of disinfectant use in cloth and bucket cleaning protocols could result in survival of vegetative bacteria, viruses, and spores, some of which

could be pathogenic and may be cross-contaminated during subsequent wiping steps. When the bucket was filled with a QAC-based disinfectant at manufacturer-recommended concentration, even in the presence of added soil, the vegetative bacterial cell load in the solution was effectively controlled to below assay enumeration limits, meaning that the QAC effectively prevented microbial survival and cross-contamination in subsequent wiping steps. On the other hand, bucket solutions containing QAC, both with and without added soil, had little efficacy against the spores in this study because a very high concentration of spores remained in those solutions and could serve as a source of cross-contamination in sequential wiping. As stated above, the MS2 data were mixed and may not be indicative of the behavior of HNV.

CONCLUSIONS

The aim of this study was to characterize the degree of cross-contamination of representative gram-positive and -negative bacteria, spores, and viruses, to large surfaces, using a traditional cloth and bucket method. We used a commercial QAC disinfectant commonly used in restaurant settings (with and without additional soil) and a PBS (no disinfectant) control. The intention was to perform the study on large surfaces that are more representative of real-world retail food-sector environments than most previous studies, which used very small surface areas.

Although the effective inactivation of vegetative bacteria by QAC disinfectants is well recognized *(6, 12)*, the current study shows that both *L. innocua* and *E. coli* were readily transferred to laminate surfaces in the absence of a disinfectant. We also demonstrated how easily *B. cereus* spores were transferred across surfaces with the cloth and bucket method, even in the presence of the QAC disinfectant with and without soil. Although the bacteriophage results were inconclusive and there are other potentially important factors not explored in our study (such as wiping pressure or the possible decreased activity of QACs when using cloth towels, which may aid the spread of organisms to surfaces *(4)*), our study does suggest that use of reusable cloths in cloth and bucket systems could potentially promote cross-contamination and recontamination of laminate surfaces. This has significance to food service establishments, schools, and many other settings. Characterizing the efficacy of other disinfectants and/or disposable wipe disinfection systems in preventing the transfer of microorganisms between surfaces, compared with the QAC cloth and bucket system evaluated in this study, would be useful in developing best practices in disinfection of large surfaces.

ACKNOWLEDGMENT

This work was possible thanks to funding from PDI, Incorporated.

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