

# Evaluation of a Disinfectant Wipe Intervention on Fomite-to-Finger Microbial Transfer

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Inanimate surfaces, or fomites, can serve as routes of transmission of enteric and respiratory pathogens. No previous studies have evaluated the impact of surface disinfection on the level of pathogen transfer from fomites to fingers. Thus, the present study investigated the change in microbial transfer from contaminated fomites to fingers following disinfecting wipe use. *Escherichia coli* ( $10^8$  to  $10^9$  CFU/ml), *Staphylococcus aureus* ( $10^9$  CFU/ml), *Bacillus thuringiensis* spores ( $10^7$  to  $10^8$  CFU/ml), and poliovirus 1 ( $10^8$  PFU/ml) were seeded on ceramic tile, laminate, and granite in 10- $\mu$ l drops and allowed to dry for 30 min at a relative humidity of 15 to 32%. The seeded fomites were treated with a disinfectant wipe and allowed to dry for an additional 10 min. Fomite-to-finger transfer trials were conducted to measure concentrations of transferred microorganisms on the fingers after the disinfectant wipe intervention. The mean  $\log_{10}$  reduction of the test microorganisms on fomites by the disinfectant wipe treatment varied from 1.9 to 5.0, depending on the microorganism and the fomite. Microbial transfer from disinfectant-wipe-treated fomites was lower (up to <0.1% on average) than from nontreated surfaces (up to 36.3% on average, reported in our previous study) for all types of microorganisms and fomites. This is the first study quantifying microbial transfer from contaminated fomites to fingers after the use of disinfectant wipe intervention. The data generated in the present study can be used in quantitative microbial risk assessment models to predict the effect of disinfectant wipes in reducing microbial exposure.

Inanimate objects and surfaces (fomites) are known to be a reservoir for the transmission of pathogens in the environment directly, by surface contact with the mouth or abraded skin, or indirectly by contamination of fingers and subsequent hand-to-mouth, hand-to-eye, or hand-to-nose contact (1, 2). Previous laboratory studies have modeled food preparation in domestic kitchens to better understand cross-contamination of food-borne pathogens (3, 4). The occurrence and spread of pathogens throughout the home and health care settings have also been studied to better understand the role of fomites in pathogen exposure and acquired infections (5–11). The potential for pathogen transfer from contaminated fomites to fingers is a concern in health care environments; particularly those in close proximity to the patient that are frequently touched (12–25). Health care-acquired infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-susceptible *S. aureus*, and *Clostridium difficile* are associated with high morbidity and mortality (18, 20, 22, 26–30). Most nosocomial and food-borne pathogens can persist on fomites for weeks or even months (18, 19, 31, 32) and on fingers for up to several hours (33–35). Pathogen presence and survival on fomites in domestic homes, public places, hospitals, and other health care facilities are important factors in evaluating potential health risks to humans (36).

Environmental and hand hygiene is crucial in preventing the spread of infectious diseases in homes, health care facilities, and public places. Numerous studies have examined the efficacy of surface cleaners and disinfectants in reducing pathogen exposure in households (7, 37, 38), hospitals (14, 16, 18, 19, 23–25, 29, 30, 37, 58–60), and nursing homes (20). However, only few studies have quantitatively assessed the efficiency of microbial transfers to and from various surfaces or the ability of disinfectant wipe intervention to inhibit such transfers. Further studies are needed for the development of quantitative microbial risk assessment (QMRA) models to assess the impact of interventions on the risk of infection (2, 39–42).

Recently, we reported fomite-to-finger transfer efficiencies of various types of microbial pathogens and fomites at different relative humidity levels (43). In the present study, we assessed the impact of a disinfectant wipe intervention on microbial transfer from contaminated fomites to fingers.

## MATERIALS AND METHODS

**Subjects.** A single subject conducted the fomite-to-finger transfer experiments. Permission was obtained from the University of Arizona's Office for Human Subjects Research prior to the study.

**Bacteria, virus, and preparation of inocula.** (i) **Study microorganisms.** *Escherichia coli* C3000 (ATCC 15597), *S. aureus* (ATCC 25923), and *Bacillus thuringiensis* (ATCC 10792) were obtained from the American Type Culture Collection (ATCC; Manassas, VA). Poliovirus 1 (PV-1; strain LSc-2ab) was obtained from the Department of Virology and Epidemiology at the Baylor College of Medicine (Houston, TX). These microorganisms were selected as models of human-pathogenic Gram-negative and Gram-positive bacteria, spore-forming bacteria, and viruses.

(ii) **Gram-negative and Gram-positive bacterial inoculum preparation.** Frozen aliquots of *E. coli* and *S. aureus* were transferred into separate flasks containing 100 to 150 ml of tryptic soy broth (TSB; EMD Chemicals Inc., Gibbstown, NJ), incubated for  $18 \pm 2$  h at 37°C on an orbital shaker (150 to 180 rpm), and streaked for isolation onto tryptic soy agar (TSA; EMD Chemicals Inc.) plates. The bacteria were then subcultured in a flask of TSB and incubated for  $18 \pm 2$  h at 37°C on an orbital shaker (150 to 180 rpm) (44).

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(iii) **Endospore-forming bacterial inoculum preparation.** *B. thuringiensis* spores were prepared as previously described, with minor modifications (45). Briefly, spores were suspended in Difco sporulation medium with supplements (DSM+S; Becton, Dickinson and Company, Sparks, MD), cultivated for 24 h at 37°C on an orbital shaker (150 to 180 rpm), and resuspended in fresh DSM+S to a final optical density of 600 nm of 0.1 (Spectronic Genesys 5; Milton Roy, Ivyland, PA).

(iv) **Virus inoculum preparation.** PV-1 propagation and plaque-forming assays were conducted as described previously (46, 47). Briefly, PV-1 was propagated on buffalo green monkey (BGM) (ATCC CCL-81; American Type Culture Collection, Manassas, VA) kidney cell line monolayers with minimal essential medium containing 5% calf serum (HyClone Laboratories, Logan, UT) at an incubation temperature of 37°C with 5% CO<sub>2</sub>. PV-1 was propagated by inoculating cell monolayers. Following the observation of ≥90% destruction of the monolayer, the cell culture flasks were frozen (at -20°C) and thawed (at 37°C) three successive times to release the viruses from the host cells. The suspension was then centrifuged (1,000 × g for 10 min) to remove cell debris; this was followed by precipitation with polyethylene glycol (9% [wt/vol]) and sodium chloride (5.8% [wt/vol]) overnight at 4°C (48). The virus was then centrifuged (15,300 × g for 30 min at 4°C). After resuspension of the virus pellet in phosphate-buffered saline (pH 7.4; Sigma-Aldrich, St. Louis, MO), Vertrel XF extraction was performed at a 1:1 ratio to promote monodispersal dispersion of the virus and the removal of lipids (centrifugation at 1,900 × g for 15 min at 4°C) (48). Plaque-forming assays were performed with six-well plates and confluent monolayers of BGM cells.

**Control wash and disinfection.** Prior to all experiments, the subject's hands were washed with warm water and nonantibacterial liquid dish detergent (Liquid Joy; Procter and Gamble, Cincinnati, OH) for 45 s, rinsed with water, and dried with paper towels. Each hand was then sprayed twice with 70% ethanol, the alcohol was rubbed thoroughly over the hands and wrists for 15 s, and the hands were subsequently air dried. After conducting fomite-to-finger transfer experiments with the prepared inocula, fingers were disinfected twice with 70% ethanol, wrapped with a 70% ethanol-saturated paper towel for 30 s, washed and rinsed with warm water and Softsoap antibacterial liquid hand soap (Colgate-Palmolive, Morristown, NJ) for 45 s, and then dried with paper towels. After the sampling of fingers for *B. thuringiensis* spores and PV-1, fingers were placed in 10% bleach (The Clorox Company, Oakland, CA) for 15 s and then neutralized with 10% sodium thiosulfate (EMD Chemicals Inc.). The hands were then washed as described above to prepare for subsequent trials. Up to four trials were performed on the same day, and no visible change in skin condition was observed throughout the day of the experiments.

**Relative humidity and temperature.** The seeded fomites were placed in an incubator; the incubator was turned off and thus was at room temperature (i.e., 19 to 25°C). The temperature and relative humidity in the incubator were monitored with a high-accuracy Thermo Hygrometer (VWR, Mississauga, Ontario, Canada). To maintain a relative humidity of 15 to 32%, t.h.e. (EMD Chemicals Inc.) and Drierite (Drierite, Xenia, OH) desiccants were used to decrease the relative humidity in the incubator when the ambient relative humidity in the laboratory was greater than 32%.

**Fomites tested.** The three nonporous fomite materials tested, ceramic tile porcelain (Home Depot, Atlanta, GA), laminate (Wilsonart International, Temple, TX), and granite (GRANITE Kitchen & Bath Countertops, Tucson, AZ), ranged in surface area from 16 to 25 cm<sup>2</sup>. After fomite-to-finger transfers of *E. coli* and *S. aureus*, the fomites were sprayed three times with 70% ethanol and allowed to dry for 10 min. Fomites used for finger transfers of *B. thuringiensis* spores and PV-1 were disinfected with 10% bleach (The Clorox Company), allowed to sit for 10 min, and subsequently neutralized in 10% sodium thiosulfate (EMD Chemicals Inc.). The fomites were then washed with warm running water and nonantibacterial soap (Liquid Joy; Procter and Gamble), rubbed with a wet

paper towel on the seeded surface area, rinsed thoroughly with reverse osmosis-treated water, air dried, and autoclaved.

**Disinfectant wipe.** The disinfectant tested in this study was a ready-to-use disinfectant wipe (Clorox Disinfecting Wipes; The Clorox Company) measuring 20.5 by 18.0 cm. The active ingredients were: *n*-alkyl (C<sub>14</sub>, 60%; C<sub>16</sub>, 30%; C<sub>12</sub>, 5%; C<sub>18</sub>, 5%) dimethyl benzyl ammonium chloride at 0.184% and *n*-alkyl (C<sub>12</sub>, 68%; C<sub>14</sub>, 32%) dimethyl ethylbenzyl ammonium chloride at 0.184%, which are quaternary ammonium compounds (QACs).

**Seeding of fomites. (i) Layout of fomites.** For each of the three nonporous fomites, 10 swatches were evenly spaced in three rows on the middle shelf of an incubator. Of the 10 swatches, 6 were subjected to application of the disinfectant wipe, of which 3 were subjected to fomite-to-finger transfer events and designated "treated-transfer" swatches, and the other 3 were not subjected to transfer events and designated "treated-nontreatment" control swatches. Three were used as "nontreated-nontreatment" control swatches (with neither the application of a disinfectant wipe nor a fomite-to-finger transfer event), and the remaining swatch was used as a negative control to ensure that fomites were not previously contaminated.

**(ii) Microorganism concentration.** The concentrations of microorganisms added to the fomites were approximately 10<sup>8</sup> to 10<sup>9</sup> CFU/ml of *E. coli* in TSB (EMD Chemicals Inc.), 10<sup>9</sup> CFU/ml of *S. aureus* in TSB, 10<sup>7</sup> to 10<sup>8</sup> CFU/ml of *B. thuringiensis* spores in DSM+S medium (Becton, Dickinson and Company), and 10<sup>8</sup> PFU/ml of PV-1 in phosphate-buffered saline (PBS; Sigma-Aldrich) in 10-μl droplets. With a pipette tip, the 10-μl droplets were spread over approximately a 1.0-cm<sup>2</sup> area in the center of each fomite. The fomites were allowed to dry for 30 min and were visibly dry in the relative humidity range.

**Fomite-to-finger transfer, sampling, intervention, and assays. (i) Fomite sampling.** The nontreated-nontreatment control swatches and the treated-nontreatment control swatches were sampled with a cotton-tipped swab applicator (Puritan Medical Products Company, Guilford, ME) after seeding with *E. coli*, *S. aureus*, and *B. thuringiensis*. In the case of PV-1, a polyester fiber-tipped applicator swab (Falcon; Becton, Dickinson and Company, Cockeysville, MD) was used. Swabs were wetted in 1.0 ml of D/E neutralizing medium (EMD Chemicals Inc.), and then an area of approximately 6 cm<sup>2</sup> on the fomite was swabbed with a firm sweeping-and-rotating motion to ensure that the entire area (approximately 1 cm<sup>2</sup>) was swabbed. The swab was then placed back into the remaining D/E and vortexed for 5 s.

**(ii) Intervention application.** The six swatches that were to be treated with the disinfectant wipe were taken one at a time from the incubator, placed on the counter, and held stationary with the left hand. Subsequently, the swatch was treated with a disinfectant wipe applied in one application and one sweeping motion in one direction (from the far corner of the swatch toward the proximal corner) in a pressure range of 300 to 500 g/cm<sup>2</sup> (22, 29, 30, 49). The application of the disinfectant wipe was approximately 1 s long, as previously described (50). The disinfectant wipe was then discarded, and the fomite was placed back into the controlled-humidity room temperature incubator for an additional 10 min of drying (50, 51).

**(iii) Transfer experiment.** One transfer trial consisted of three separate fomite-to-finger transfer events with the index, middle, and ring fingers of the right hand for each surface type. Two transfer trials were conducted, resulting in a total of six transfers for each fomite. A protocol from Ansari et al. (33) and Mbithi et al. (34) was used to perform the transfer experiments with modifications. Briefly, the fomite-to-finger transfer experiments were performed after 30 min of inoculum drying plus an additional 10 min of drying after application of the disinfectant wipe. The following conditions were used for the transfer experiments. The fomite was placed at the center of a scale with a digital readout, and a finger transfer was performed by placing the right-hand finger on the center, covering the seeded area of the fomite for 10 s with an average pressure of 1.0 kg/cm<sup>2</sup> (98.0665 kPa; range, of 700 to 1,500 g/cm<sup>2</sup>) (33, 34).

TABLE 1 Microorganisms recovered from treated-nontransfer and nontreated-nontransfer fomites

Surface type or parameter	Mean count $\pm$ SD (range)			
	<i>E. coli</i>	<i>S. aureus</i>	<i>B. thuringiensis</i>	PV-1
Ceramic tile				
Nontreated <sup>a</sup>	5.1 $\pm$ 0.4 (4.8–5.5)	6.8 $\pm$ 0.1 (6.7–6.8)	4.6 $\pm$ 0.2 (4.4–4.8)	5.6 $\pm$ 0.4 (5.1–6.2)
Treated <sup>a</sup>	<1.6 $\pm$ 0.9 (<1.0–3.2) <sup>b</sup>	2.3 $\pm$ 0.6 (1.5–2.9)	2.7 $\pm$ 0.4 (2.2–3.4)	1.6 $\pm$ 0.4 (1.0–2.1)
Reduction <sup>c</sup>	3.6 $\pm$ 0.9 (2.3–>4.5)	4.5 $\pm$ 0.6 (3.7–5.3)	1.9 $\pm$ 0.5 (1.1–2.5)	3.9 $\pm$ 0.8 (3.0–>5.2)
Laminate				
Nontreated <sup>a</sup>	5.1 $\pm$ 0.9 (4.2–6.6)	6.9 $\pm$ 0.1 (6.8–7.1)	4.6 $\pm$ 0.1 (4.5–4.7)	5.6 $\pm$ 0.1 (5.5–5.7)
Treated <sup>a</sup>	<1.2 $\pm$ 0.4 (<1.0–1.9) <sup>b</sup>	<1.9 $\pm$ 0.8 (<1.0–2.9) <sup>b</sup>	<2.1 $\pm$ 0.6 (<1.0–2.6) <sup>b</sup>	<1.8 $\pm$ 0.5 (<1.0–2.5) <sup>b</sup>
Reduction <sup>c</sup>	4.0 $\pm$ 0.9 (3.2–>5.6)	5.0 $\pm$ 0.8 (4.0–>5.8)	2.5 $\pm$ 0.7 (1.9–>3.6)	3.8 $\pm$ 0.5 (3.2–>4.7)
Granite				
Nontreated <sup>a</sup>	5.4 $\pm$ 0.1 (5.3–5.6)	6.8 $\pm$ 0.3 (6.4–7.1)	4.5 $\pm$ 0.3 (4.1–4.9)	5.5 $\pm$ 0.2 (5.0–5.7)
Treated <sup>a</sup>	<1.9 $\pm$ 1.0 (<1.0–3.2) <sup>b</sup>	<2.5 $\pm$ 1.2 (<1.0–3.8) <sup>b</sup>	<2.4 $\pm$ 0.8 (<1.0–3.2) <sup>b</sup>	<1.8 $\pm$ 0.8 (<1.0–2.8) <sup>b</sup>
Reduction <sup>c</sup>	3.5 $\pm$ 0.9 (2.4–>4.3)	4.4 $\pm$ 1.1 (3.1–>5.7)	2.1 $\pm$ 0.9 (1.3–>3.8)	3.7 $\pm$ 0.9 (2.8–>4.5)

<sup>a</sup> Values are expressed as log<sub>10</sub> numbers of CFU/2 cm<sup>2</sup> or PFU/2 cm<sup>2</sup> ( $n = 6$  for each fomite and microorganism).

<sup>b</sup> Transfer of organisms from fomites to fingers for one or more transfer events were below the detection limit of 10 CFU/2 cm<sup>2</sup> (indicated by a less-than sign).

<sup>c</sup> Log<sub>10</sub> reduction =  $-\log_{10}(\text{CFU or PFU control fomite with intervention}/\text{CFU or PFU control fomite without intervention})$ .

(iv) **Finger sampling.** With a cotton-tipped swab applicator (Puritan Medical Products Company) wetted in 1.0 ml of D/E neutralizing medium (EMD Chemicals Inc.), the index, middle, and ring finger pads were sampled with a sweeping-and-rotating motion. Subsequently, the swab applicator was placed in the D/E vial and vortexed. A polyester-tipped swab (Puritan Medical Products Company) was used to sample PV-1.

(v) **Microorganism assays.** *E. coli*, *S. aureus*, and *B. thuringiensis* spores were enumerated by the spread plate technique on MacConkey agar (EMD Chemicals Inc.), mannitol salt agar (EMD Chemicals Inc.), and TSA (EMD Chemicals Inc.) plates, respectively. The plates were incubated at 37°C for 18  $\pm$  2 h. *B. thuringiensis* spore samples were heat shocked at 81  $\pm$  2°C for 10 min prior to spread plating to stimulate germination. PV-1 titrations were performed by using 10-fold serial dilution plaque-forming assays as described previously (46, 47). All dilutions were assayed in duplicate. If no bacteria or virus was recovered from the finger pad, the lower detection limit of 10 CFU or PFU was used to estimate the number of microorganisms recovered, as previously described (52, 53). The swabbed microorganisms were eluted in 1,000  $\mu$ l of PBS, of which 100  $\mu$ l was used for plate counting (bacteria) or plaque assay (virus). Assuming that the detection limit is 1 CFU or PFU per assay, the lower detection limit was 10 CFU or PFU/2 cm<sup>2</sup>.

**Calculations and statistical analyses.** (i) **Calculation formulae.** The “resulting transfer” was defined as microorganism transfer to the finger, regardless of the application of the disinfectant wipe, relative to the recovered microorganisms from the nontreated-nontransfer control fomite, which is expressed by equation 1:

$$\text{resulting transfer (\%)} = \left( \frac{\text{CFU or PFU on finger}}{\text{CFU or PFU on nontreated-nontransfer control fomite}} \right) \times 100 \quad (1)$$

A resulting transfer value with a less-than sign indicates that the number of microorganisms on the finger was lower than the lower limit of detection. Log<sub>10</sub> microbial count reduction on fomites following disinfectant wipe use was calculated with equation 2:

$$\text{reduction (log}_{10}\text{)} = -\log_{10}\left( \frac{\text{CFU or PFU on treated fomite}}{\text{CFU or PFU on nontreated fomite}} \right) \quad (2)$$

where the “treated fomite” represents the disinfectant-wipe-treated non-transfer control fomite.

(ii) **Statistical analyses.** Data were entered in Microsoft Excel 2010 (Microsoft Corp., Redmond, WA) and the software package StatPlus:mac 2009 (AnalystSoft) to compute the descriptive statistic measures of mean percent resulting transfer, the standard deviation, and statistical signifi-

cance. Student’s *t* test was performed to determine whether there was a statistically significant difference in percent resulting transfer of a particular type of microorganism between the disinfectant-wipe-treated and nontreated fomites. Differences were considered statistically significant if the resultant *P* value was 0.05 or lower.

## RESULTS

**Removal of microorganisms from fomites by the disinfectant wipe.** To determine the sources of transfer potential with and without intervention, microbial counts of *E. coli*, *S. aureus*, *B. thuringiensis* spores, and PV-1 recovered from disinfectant-treated–nontransfer control fomites were compared to those recovered from nontreated-nontransfer control fomites. The numbers of test microorganisms recovered from nontreated-nontransfer fomites (4.5 to 6.9 log<sub>10</sub> CFU/2 cm<sup>2</sup> or PFU/2 cm<sup>2</sup>) were significantly greater than those recovered from treated-nontransfer fomites (<1.2 to 2.7 log<sub>10</sub> CFU/2 cm<sup>2</sup> or PFU/2 cm<sup>2</sup>) for all types of microorganisms and fomites tested (Student’s *t* test, *P* < 0.05), resulting in mean log<sub>10</sub> microbial reductions of 1.9 to 5.0 (Table 1). Reduction of *B. thuringiensis* spores was always less than that of the other microorganisms (mean log<sub>10</sub> reduction of 1.9 to 2.5 versus 3.5 to 5.0) (Table 1), showing that *B. thuringiensis* spores were more likely to be available for transfer after disinfectant wipe treatment.

**Disinfectant wipe intervention effect on fomite-to-finger microbial transfer.** In order to evaluate the intervention effect on fomite-to-finger microbial transfer, we compared the resulting transfer from the disinfectant-wipe-treated fomite with that from the nontreated fomite; the resulting transfer from the nontreated fomites was adapted from our previous study (43) (Table 2). The resulting transfer from the disinfectant-wipe-treated fomites was lower than from the nontreated fomites for all types of microorganisms and fomites tested (mean value of <0.0002 to 0.1% versus <0.04 to 36.3%) (Table 2). The counts of *E. coli* CFU on the finger were all below the detection limit of 10 CFU/cm<sup>2</sup> for all disinfectant-wipe-treated fomites, which produced a less-than percent resulting transfer value for all trials; the other microorganisms also showed a less-than percent resulting transfer value for at least one trial.

TABLE 2 Resulting fomite-to-finger microorganism transfer

Surface type	Avg % transfer efficiency $\pm$ SD (range) <sup>a</sup>			
	<i>E. coli</i>	<i>S. aureus</i>	<i>B. thuringiensis</i>	PV-1
Ceramic tile				
Nontreated <sup>d</sup>	11.6 $\pm$ 11.8 (0.1–33.3)	2.7 $\pm$ 2.3 (0.8–6.7)	<0.2 $\pm$ 0.1 (<0.1–0.4) <sup>b</sup>	23.1 $\pm$ 24.0 (0.4–52.7)
Treated	<0.01 (<0.02) <sup>b</sup>	<0.003 $\pm$ 0.01 (<0.0001–0.02) <sup>b</sup>	<0.04 $\pm$ 0.03 (<0.02–0.1) <sup>b</sup>	<0.01 $\pm$ 0.02 (<0.004–0.05) <sup>b</sup>
Laminate				
Nontreated <sup>d</sup>	21.7 $\pm$ 23.9 (5.2–66.5)	4.3 $\pm$ 2.4 (1.3–7.4)	<0.2 $\pm$ 0.1 (<0.1–0.3) <sup>b</sup>	36.3 $\pm$ 8.7 (24.1–50.0)
Treated	<0.02 (<0.1) <sup>b</sup>	<0.0002 $\pm$ 0.0001 (<0.0001–0.0004) <sup>b</sup>	<0.03 (<0.03) <sup>b</sup>	<0.004 $\pm$ 0.004 (<0.002–0.01) <sup>b</sup>
Granite				
Nontreated <sup>d</sup>	<7.3 $\pm$ 10.6 (<0.1–28.0) <sup>b</sup>	3.9 $\pm$ 5.0 (0.7–13.9)	<0.04 $\pm$ 0.03 (<0.02–0.1) <sup>b</sup>	33.8 $\pm$ 40.4 (0.4–100) <sup>c</sup>
Treated	<0.004 (<0.01) <sup>b</sup>	<0.001 $\pm$ 0.001 (<0.0002–0.004) <sup>b</sup>	<0.1 $\pm$ 0.1 (<0.01–0.2) <sup>b</sup>	<0.01 $\pm$ 0.01 (<0.002–0.02) <sup>b</sup>

<sup>a</sup> Percent transfer efficiency = (CFU or PFU finger/CFU or PFU control fomite)  $\times$  100 ( $n$  = 6 for each fomite and microorganism).

<sup>b</sup> Transfer of organisms from fomites to fingers for one or more transfer events was below the detection limit of 10 CFU/2 cm<sup>2</sup> (indicated by a less-than sign).

<sup>c</sup> Transfer of organisms from fomites to fingers in one or more transfer events was >100% and was truncated to 100%.

<sup>d</sup> The resulting transfer from the nontreated fomites was adapted from our previous study (43).

The microorganism counts on treated-nontransfer control fomites were significantly low, because the disinfectant wipe was highly effective at removing microorganisms from the fomites or inactivating them on the fomites (Table 1). As a result, we were unable to recover microorganisms from fingers for many replicates, which produced less-than percent resulting transfer values for all types of microorganisms and fomites (Table 2).

## DISCUSSION

The purpose of the present study was to determine the effect of a disinfectant wipe intervention on microbial transfer from contaminated fomites to fingers. While laboratory protocols to determine the efficacy of disinfectant wipes on microbial reduction are available, no empirical studies have been conducted to determine the impact of disinfectant wipe treatment on potential microbial transfer from fomites to fingers. The information obtained in the present study can be used to model the probability of infection in disinfectant-wipe-treated and nontreated scenarios and assess microbial exposure via fomites. However, the lack of standard methods for quantifying microbial transfer makes it difficult to compare the results of different studies (43). In the present study, we used the same experimental parameters as in our previous study (43), such as the humidity level, fomite-to-finger contact pressure, drying time, sampling method, etc., which allowed us to compare the fomite-to-finger microbial transfer values from both studies. In order to directly compare the transferred microbial numbers relative to the original microbial numbers on the fomite between treated and nontreated conditions, we used the “resulting transfer” defined by equation 1. Using the resulting transfer enabled us to show the effect of the disinfectant wipe on microbial transfer to fingers. In addition, including more volunteer hands could also help to better characterize the variability that might be expected among different individuals. However, the main focus of the present study was not to determine the variability of fomite-to-finger microbial transfer that results from different subjects but rather to obtain a clearer picture of the effect of the disinfectant wipe on microbial transfer to fingers. The variability of fomite-to-finger microbial transfer efficiency among different subjects needs to be investigated in future studies.

Four types of microorganisms, *E. coli*, *S. aureus*, *B. thuringiensis*, and PV-1, were used in the present study as models. These

microorganisms have been widely used as models of Gram-positive and -negative bacteria, spore-forming bacteria, and enteric viruses. Especially *E. coli* and *S. aureus* have been used in transfer studies (43, 54–56).

In the present study, a 10-min drying time was used following the application of a disinfectant wipe to allow the treated fomites to dry, as described previously (50, 51). During the 10-min drying time, the active ingredients (i.e., QACs) might have interacted with any remaining microorganisms after physical removal by the wipe, which may produce an additional microbial count reduction (50, 51); QACs are known to damage bacterial cell membranes, but their effect against bacterial spores is limited (57).

As expected, our results showed that the use of a disinfectant wipe greatly reduced the microbial load on fomites (Table 1). *S. aureus*, *E. coli*, and PV-1 counts on fomites were reduced by 3.5 to 5.0 log<sub>10</sub>, whereas *B. thuringiensis* spore counts were reduced by up to only 2.5 log<sub>10</sub>. These findings are consistent with previous studies that showed that disinfectant wipes reduce the microbial load on contaminated surfaces (22, 29, 30, 49–51). The present study demonstrated *B. thuringiensis* spores to be more resistant to physical removal and/or inactivation by disinfectant wipes than non-spore-forming bacteria, which is consistent with the findings of Siani et al. (22) for *Clostridium difficile* spores. Panousi et al. (49) on the other hand, showed *B. subtilis* spores to be more readily removed from surfaces by impregnated wipes than *Staphylococcus epidermidis* and MRSA.

We conducted these experiments under clean-fomite conditions in order to minimize the variability of fomite surface conditions, which reflect the same experimental conditions as our previous study (43), but a previous study was conducted showing the efficacy of disinfectant wipes under dirty- and clean-surface conditions and reported a similar mean log<sub>10</sub> removal (30). However, an earlier study suggests that the presence of a higher organic material on surfaces can reduce the ability of the wipes to remove bacteria (29). Several studies have tested the efficacy of disinfectant wipes by the method described by Williams et al. (29) and found results similar to those of the present study, showing microbial reduction on fomites after wiping with a disinfectant wipe (22, 29, 30, 49). In addition, our findings are also consistent with previous studies that observed a microbial count reduction after

surface wiping one to five times with various types of disinfectant wipes and under a different study design (50, 51). Further studies are still needed to evaluate the use of disinfectant wipes under different specific conditions, such as dirty or clean surfaces, different microbial loads, different contact pressures, and different contact times in order to better understand how these conditions influence fomite-to-finger transfer.

To our knowledge, this is the first study that has quantified microbial transfer from contaminated fomites to fingers after the use of disinfectant wipes as an intervention. Our results show the impact of disinfectant wipes in reducing the resulting transfer of microorganisms and help to predict the benefit of interventions without collecting additional observational data. The use of the resulting transfer data that were generated in the present study can improve model accuracy where this information could not be previously incorporated in QMRA models to predict the ability of disinfectant wipes to reduce microbial exposure.

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## REFERENCES

- Haas CN, Rose JB, Gerba CP. 1999. Quantitative microbial risk assessment. John Wiley, New York, NY.
- Nicas M, Best D. 2008. A study quantifying the hand-to-face contact rate and its potential application to predicting respiratory tract infection. *J. Occup. Environ. Hyg.* 5:347–352. <http://dx.doi.org/10.1080/15459620802003896>.
- van Asselt ED, De Jong AEI, De Jonge R, Nauta MJ. 2008. Cross-contamination in the kitchen: estimation of transfer rates for cutting boards, hands and knives. *J. Appl. Microbiol.* 105:1392–1401. <http://dx.doi.org/10.1111/j.1365-2672.2008.03875.x>.
- de Jong AEI, Verhoeff-Bakkenes L, Nauta MJ, De Jonge R. 2008. Cross-contamination in the kitchen: effect of hygiene measures. *J. Appl. Microbiol.* 105:615–624. <http://dx.doi.org/10.1111/j.1365-2672.2008.03778.x>.
- Fekety R, Kyung-Hee K, Brown D, Batts DH, Cudmore M, Silva J. 1981. Epidemiology of antibiotic-associated colitis: isolation of *Clostridium difficile* from the hospital environment. *Am. J. Med.* 70:906–908. [http://dx.doi.org/10.1016/0002-9343\(81\)90553-2](http://dx.doi.org/10.1016/0002-9343(81)90553-2).
- Josephson KL, Rubino JR, Pepper IL. 1997. Characterization and quantification of bacterial pathogens and indicator organisms in household kitchens with and without the use of a disinfectant cleaner. *J. Appl. Microbiol.* 83:737–750. <http://dx.doi.org/10.1046/j.1365-2672.1997.00308.x>.
- Medrano-Félix A, Martínez C, Castro-del Campo N, León-Félix J, Peraza-Garay F, Gerba CP, Chaidez C. 2011. Impact of prescribed cleaning and disinfectant use on microbial contamination in the home. *J. Appl. Microbiol.* 110:463–471. <http://dx.doi.org/10.1111/j.1365-2672.2010.04901.x>.
- Rusin P, Orosz-Coughlin P, Gerba C. 1998. Reduction of faecal coliform, coliform and heterotrophic plate count bacteria in the household kitchen and bathroom by disinfection with hypochlorite cleaners. *J. Appl. Microbiol.* 85:819–828. <http://dx.doi.org/10.1046/j.1365-2672.1998.00598.x>.
- Scott E, Bloomfield SF, Barlow CG. 1982. An investigation of microbial contamination in the home. *J. Hyg. (Lond.)* 89:279–293.
- Sinclair RG, Gerba CP. 2011. Microbial contamination in kitchens and bathrooms of rural Cambodian village households. *Lett. Appl. Microbiol.* 52:144–149. <http://dx.doi.org/10.1111/j.1472-765X.2010.02978.x>.
- Speirs JP, Anderton A, Anderson JG. 1995. A study of the microbial content of the domestic kitchen. *Int. J. Environ. Health Res.* 5:109–122. <http://dx.doi.org/10.1080/09603129509356839>.
- Bonten MJM, Hayden MK, Nathan C, van Voorhis J, Matushek M, Slaughter S, Rice T, Weinstein RA. 1996. Epidemiology of colonisation of patients and environment with vancomycin-resistant enterococci. *Lancet* 348:1615–1619. [http://dx.doi.org/10.1016/S0140-6736\(96\)02331-8](http://dx.doi.org/10.1016/S0140-6736(96)02331-8).
- Boyce JM, Potter-Bynoe G, Chenevert C, King T. 1997. Environmental contamination due to methicillin-resistant *Staphylococcus aureus*: possible infection control implications. *Infect. Control Hosp. Epidemiol.* 18:622–627. <http://dx.doi.org/10.1086/647686>.
- Hayden MK, Bonten MJM, Blom DW, Lyle EA, van de Vijver DAMC, Weinstein RA. 2006. Reduction in acquisition of vancomycin-resistant *Enterococcus* after enforcement of routine environmental cleaning measures. *Clin. Infect. Dis.* 42:1552–1560. <http://dx.doi.org/10.1086/503845>.
- Ray AJ, Hoyen CK, Taub TF, Eckstein EC, Donskey CJ. 2002. Nosocomial transmission of vancomycin-resistant enterococci from surfaces. *JAMA* 287:1400–1401. <http://dx.doi.org/10.1001/jama.287.11.1400>.
- Bhalla A, Pultz N, Gries D, Ray A, Eckstein E, Aron D, Donskey C. 2004. Acquisition of nosocomial pathogens on hands after contact with environmental surfaces near hospitalized patients. *Infect. Control Hosp. Epidemiol.* 25:164–167. <http://dx.doi.org/10.1086/502369>.
- Weber DJ, Rutala WA. 2011. The role of the environment in transmission of *Clostridium difficile* infection in healthcare facilities. *Infect. Control Hosp. Epidemiol.* 32:207–209. <http://dx.doi.org/10.1086/658670>.
- Weber DJ, Rutala WA, Miller MB, Huslage K, Sickbert-Bennett E. 2010. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, *Clostridium difficile*, and *Acinetobacter* species. *Am. J. Infect. Control* 38:S25–S33. <http://dx.doi.org/10.1016/j.ajic.2010.04.196>.
- Kramer A, Schwebke I, Kampf G. 2006. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect. Dis.* 6:130. <http://dx.doi.org/10.1186/1471-2334-6-130>.
- Murphy CR, Eells SJ, Quan V, Kim D, Peterson E, Miller LG, Huang SS. 2012. Methicillin-resistant *Staphylococcus aureus* burden in nursing homes associated with environmental contamination of common areas. *J. Am. Geriatr. Soc.* 60:1012–1018. <http://dx.doi.org/10.1111/j.1532-5415.2012.03978.x>.
- Sexton JD, Tanner BD, Maxwell SL, Gerba CP. 2011. Reduction in the microbial load on high-touch surfaces in hospital rooms by treatment with a portable saturated steam vapor disinfection system. *Am. J. Infect. Control* 39:655–662. <http://dx.doi.org/10.1016/j.ajic.2010.11.009>.
- Siani H, Cooper C, Maillard J-Y. 2011. Efficacy of sporicidal wipes against *Clostridium difficile*. *Am. J. Infect. Control* 39:212–218. <http://dx.doi.org/10.1016/j.ajic.2011.01.006>.
- Dancer SJ. 2009. The role of environmental cleaning in the control of hospital-acquired infection. *J. Hosp. Infect.* 73:378–385. <http://dx.doi.org/10.1016/j.jhin.2009.03.030>.
- Dancer SJ, White L, Robertson C. 2008. Monitoring environmental cleanliness on two surgical wards. *Int. J. Environ. Health Res.* 18:357–364. <http://dx.doi.org/10.1080/09603120802102465>.
- White LF, Dancer SJ, Robertson C, McDonald J. 2008. Are hygiene standards useful in assessing infection risk? *Am. J. Infect. Control* 36:381–384. <http://dx.doi.org/10.1016/j.ajic.2007.10.015>.
- Cosgrove S, Qi Y, Kaye K, Harbarth S, Karchmer A, Carmeli Y. 2005. The impact of methicillin resistance in *Staphylococcus aureus* bacteremia on patient outcomes: mortality, length of stay, and hospital charges. *Infect. Control Hosp. Epidemiol.* 26:166–174. <http://dx.doi.org/10.1086/502522>.
- Humphreys H. 2009. Do guidelines for the prevention and control of methicillin-resistant *Staphylococcus aureus* make a difference? *Clin. Microbiol. Infect.* 15:39–43. <http://dx.doi.org/10.1111/j.1469-0691.2009.03095.x>.
- Schweickert B, Geffers C, Farragher T, Gastmeier P, Behnke M, Eckmanns T, Schwab F. 2011. The MRSA-import in ICUs is an important predictor for the occurrence of nosocomial MRSA cases. *Clin. Microbiol. Infect.* 17:901–906. <http://dx.doi.org/10.1111/j.1469-0691.2010.03409.x>.
- Williams GJ, Denyer SP, Hosein IK, Hill DW, Maillard JY. 2007. The development of a new three-step protocol to determine the efficacy of disinfectant wipes on surfaces contaminated with *Staphylococcus aureus*. *J. Hosp. Infect.* 67:329–335. <http://dx.doi.org/10.1016/j.jhin.2007.08.012>.
- Williams GJ, Denyer SP, Hosein IK, Hill DW, Maillard J-Y. 2009. Limitations of the efficacy of surface disinfection in the healthcare setting. *Infect. Control Hosp. Epidemiol.* 30:570–573. <http://dx.doi.org/10.1086/597382>.
- Masago Y, Shibata T, Rose JB. 2008. Bacteriophage P22 and *Staphylococcus aureus* attenuation on nonporous fomites as determined by plate assay and quantitative PCR. *Appl. Environ. Microbiol.* 74:5838–5840. <http://dx.doi.org/10.1128/AEM.00352-08>.
- Neely AN, Maley MP. 2000. Survival of enterococci and staphylococci on hospital fabrics and plastic. *J. Clin. Microbiol.* 38:724–726. [http://dx.doi.org/10.1016/S0001-2092\(06\)61994-7](http://dx.doi.org/10.1016/S0001-2092(06)61994-7).
- Ansari SA, Sattar SA, Springthorpe VS, Wells GA, Tostowaryk W. 1988. Rotavirus survival on human hands and transfer of infectious virus to animate and nonporous inanimate surfaces. *J. Clin. Microbiol.* 26:1513–1518.
- Mbithi JN, Springthorpe VS, Boulet JR, Sattar SA. 1992. Survival of

- hepatitis A virus on human hands and its transfer on contact with animate and inanimate surfaces. *J. Clin. Microbiol.* 30:757–763.
35. von Rheinbaben F, Schunemann S, Grob T, Wolff MH. 2000. Transmission of viruses via contact in a household setting: experiments using bacteriophage ΦX174 as a model virus. *J. Hosp. Infect.* 46:61–66. <http://dx.doi.org/10.1053/jhin.2000.0794>.
  36. Reynolds KA, Watt PM, Boone SA, Gerba CP. 2005. Occurrence of bacteria and biochemical markers on public surfaces. *Int. J. Environ. Health Res.* 15:225–234. <http://dx.doi.org/10.1080/09603120500115298>.
  37. Bloomfield S, Exner M, Fara GM, Scott EA. 2008. Prevention of the spread of infection: the need for a family-centered approach to hygiene education. *Euro Surveill.* 13:18889. <http://dx.doi.org/10.1016/j.ajic.2009.11.001>.
  38. Scott E, Bloomfield SF, Exner M, Fara G, Nath K, Signorelli C, Van der Voorden C. 2010. Prevention of the spread of infection: the need for a family-centered approach to hygiene promotion. *Am. J. Infect. Control* 38:1–2. <http://dx.doi.org/10.1016/j.ajic.2009.11.001>.
  39. Atkinson M, Wein L. 2008. Quantifying the routes of transmission for pandemic influenza. *Bull. Math. Biol.* 70:820–867. <http://dx.doi.org/10.1007/s11538-007-9281-2>.
  40. Brankston G, Gitterman L, Hirji Z, Lemieux C, Gardam M. 2007. Transmission of influenza A in human beings. *Lancet Infect. Dis.* 7:257–265. [http://dx.doi.org/10.1016/S1473-3099\(07\)70029-4](http://dx.doi.org/10.1016/S1473-3099(07)70029-4).
  41. Boone SA, Gerba CP. 2007. Significance of fomites in the spread of respiratory and enteric viral disease. *Appl. Environ. Microbiol.* 73:1687–1696. <http://dx.doi.org/10.1128/AEM.02051-06>.
  42. Gerba CP, Rose JB, Haas CN. 1996. Sensitive populations: who is at the greatest risk? *Int. J. Food Microbiol.* 30:113–123. [http://dx.doi.org/10.1016/0168-1605\(96\)00996-8](http://dx.doi.org/10.1016/0168-1605(96)00996-8).
  43. Lopez GU, Gerba C, Tamimi A, Kitajima M, Maxwell S, Rose JB. 2013. Transfer efficiency of bacteria and viruses from porous and nonporous fomites to fingers under different relative humidity conditions. *Appl. Environ. Microbiol.* 79:5728–5734. <http://dx.doi.org/10.1128/AEM.01030-13>.
  44. Rusin P, Maxwell S, Gerba C. 2002. Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. *J. Appl. Microbiol.* 93:585–592. <http://dx.doi.org/10.1046/j.1365-2672.2002.01734.x>.
  45. Nicholson W, Setlow P. 1990. *Molecular biological methods for Bacillus*. John Wiley, New York, NY.
  46. Ikner LA, Soto-Beltran M, Bright KR. 2011. New method using a positively charged microporous filter and ultrafiltration for concentration of viruses from tap water. *Appl. Environ. Microbiol.* 77:3500–3506. <http://dx.doi.org/10.1128/AEM.02705-10>.
  47. Bidawid S, Malik N, Adegbunrin O, Sattar SS, Farber JM. 2003. A feline kidney cell line-based plaque assay for feline calicivirus, a surrogate for Norwalk virus. *J. Virol. Methods* 107:163–167. [http://dx.doi.org/10.1016/S0166-0934\(02\)00214-8](http://dx.doi.org/10.1016/S0166-0934(02)00214-8).
  48. Black S, Thurston JL, Gerba CP. 2009. Determination of  $C_T$  values for chlorine resistant enteroviruses. *J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng.* 44:336–339. <http://dx.doi.org/10.1080/10934520802659653>.
  49. Panousi MN, Williams GJ, Girdlestone S, Maillard J-Y. 2009. Use of alcoholic wipes during aseptic manufacturing. *Lett. Appl. Microbiol.* 48:648–651. <http://dx.doi.org/10.1111/j.1472-765X.2009.02574.x>.
  50. Berendt AE, Turnbull L, Spady D, Rennie R, Forgie SE. 2011. Three swipes and you're out: how many swipes are needed to decontaminate plastic with disposable wipes? *Am. J. Infect. Control* 39:442–443. <http://dx.doi.org/10.1016/j.ajic.2010.08.014>.
  51. Rutala WA, White MS, Gergen MF, Weber DJ. 2006. Bacterial contamination of keyboards: efficacy and functional impact of disinfectants. *Infect. Control. Hosp. Epidemiol.* 27:372–377. <http://dx.doi.org/10.1086/503340>.
  52. Chen Y, Jackson KM, Chea FP, Schaffner DW. 2001. Quantification and variability analysis of bacterial cross-contamination rates in common food service tasks. *J. Food Prot.* 64:72–80.
  53. Julian TR, Leckie JO, Boehm AB. 2010. Virus transfer between fingerpads and fomites. *J. Appl. Microbiol.* 109:1868–1874. <http://dx.doi.org/10.1111/j.1365-2672.2010.04814.x>.
  54. MacKintosh CA, Hoffman PN. 1984. An extended model for transfer of micro-organisms via the hands: differences between organisms and the effect of alcohol disinfection. *J. Hyg. (Lond.)* 92:345–355. <http://dx.doi.org/10.1017/S0022172400064561>.
  55. Scott E, Bloomfield SF. 1990. The survival and transfer of microbial contamination via cloths, hands and utensils. *J. Appl. Bacteriol.* 68:271–278. <http://dx.doi.org/10.1111/j.1365-2672.1990.tb02574.x>.
  56. Hubner N-O, Hubner C, Kramer A, Assadian O. 2011. Survival of bacterial pathogens on paper and bacterial retrieval from paper to hands: preliminary results. *Am. J. Nurs.* 111:30–34. <http://dx.doi.org/10.1097/01.NAJ.0000408181.37017.82>.
  57. McDonnell G, Russell AD. 1999. Antiseptics and disinfectants: activity, action, and resistance. *Clin. Microbiol. Rev.* 12:147–179. (Erratum, 14:227, 2001.)
  58. Boyce JM, Havill NL, Lipka A, Havill H, Rizvani R. 2010. Variations in hospital daily cleaning practices. *Infect. Control Hosp. Epidemiol.* 31:99–101. <http://dx.doi.org/10.1086/649225>.
  59. Mayfield JL, Leet T, Miller J, Mundy LM. 2000. Environmental control to reduce transmission of *Clostridium difficile*. *Clin. Infect. Dis.* 31:995–1000. <http://dx.doi.org/10.1086/318149>.
  60. Muto CA, Jernigan JA, Ostrowsky BE, Richet HM, Jarvis WR, Boyce JM, Farr BM. 2003. SHEA guideline for preventing nosocomial transmission of multidrug, resistant strains of *Staphylococcus aureus* and *Enterococcus*. *Infect. Control Hosp. Epidemiol.* 24:362–386. <http://dx.doi.org/10.1086/502213>.