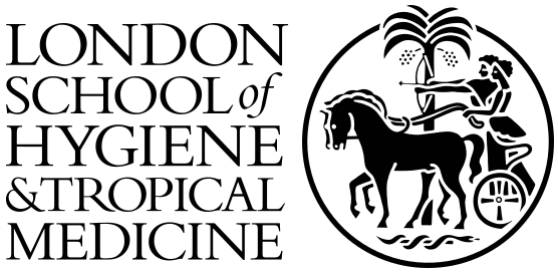

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Surface Cleaning and Disinfection: Efficacy Assessment of Four Chlorine Types Using *Escherichia coli* and the Ebola Surrogate Phi6

Karin Gallandat,*[,†] Marlene K. Wolfe,[†] and Daniele Lantagne[†]

[†]Department of Civil and Environmental Engineering, Tufts University, Medford, Massachusetts 02155, United States

ABSTRACT: In the 2014 West African Ebola outbreak, international organizations provided conflicting recommendations for disinfecting surfaces contaminated by uncontrolled patient spills. We compared the efficacy of four chlorine solutions (sodium hypochlorite, sodium dichloroisocyanurate, high-test hypochlorite, and generated hypochlorite) for disinfection of three surface types (stainless steel, heavy-duty tarp, and nitrile) with and without pre-cleaning practices (prewiping, covering, or both) and soil load. The test organisms were *Escherichia coli* and the Ebola surrogate Phi6. All tests achieved a minimum of 5.9 and 3.1 log removal in *E. coli* and Phi6, respectively. A 15 min exposure to 0.5% chlorine was sufficient to ensure <8 Phi6 plaque-forming unit (PFU)/cm^2 in all tests. While chlorine types were equally efficacious with and without soil load, variation was seen by surface type. Wiping did not increase disinfection efficacy and is not recommended because it generates infectious waste. Covering spills decreased disinfection efficacy against *E. coli* on heavy-duty tarp but does prevent splashing, which is critical in Ebola contexts. Our results support the recommendation of a 15 min exposure to 0.5% chlorine, independently of chlorine type, surface, pre-cleaning practices, and organic matter, as an efficacious measure to interrupt disease transmission from uncontrolled spills in Ebola outbreaks.

INTRODUCTION

The Ebola virus is a filamentous, enveloped, single-stranded RNA virus belonging to the Filoviridae family that was isolated in 1977 following an outbreak in Zaire (now the Democratic Republic of Congo).[1] The 2014 West African Ebola Virus Disease (EVD) outbreak was the first widespread outbreak and the largest to date, with over 28 000 cases and 11 000 deaths.[2] EVD is characterized by fever, headache, muscle pain, weakness and fatigue, diarrhea, vomiting, abdominal pain, and, in some cases, hemorrhage.[3,4]

The Ebola virus enters the host via mucosa or breaks in the skin.[5] Ebola is primarily transmitted through direct contact with an infected person or dead body, particularly when caring for a patient in the late stages of the disease and during unsafe burials. Contact with fomites (objects) or surfaces contaminated with bodily fluids also carries some transmission risk.[6−8] Recent research has found that the Ebola Makona-C05 variant, isolated during the 2014 EVD outbreak, is more resistant to drying in blood than the Yambuku-Mayinga variant from 1976, thus potentially increasing the risk of transmission via fomites.[9] This was confirmed when Ebola viral RNA was detected in multiple samples from surfaces that were not visibly bloody or soiled in an Ebola treatment unit (ETU) in Sierra Leone in 2014 and in 16 samples from bedsides in another ETU, also in Sierra Leone.[10,11] In general, the risk of disease transmission via fomites depends on[12] (1) the amount of infective viruses shed by infected individuals, (2) the infectious dose, (3) the persistence of viruses on surfaces, and (4) the resistance of viruses to surface disinfection.

In EVD patients, infective virus has been found in saliva, breast milk, and semen, and viral RNA was detected in skin and vaginal swabs, stool, tears, and nasal blood.[13−15] EVD patients may generate up to 9 L of liquid waste per day,[16] with viral RNA concentrations as high as 10^8 copies per mL in blood, 10^7 copies per mL in stool, and 10^5 copies per mL in urine.[17] In ETUs, patients will typically shed contaminated bodily fluids in dedicated buckets or latrines ("controlled spills"), as well as on other surfaces ("uncontrolled spills").[18−20] The infectious dose of viral hemorrhagic fevers such as Ebola is thought to be as low as 1−10 viral particles.[21] Infective Ebola virus was detected for 3 days on Tyvek in tropical conditions,[22] and a mean half-life of 25 h was reported for the Ebola virus Makona-C05 variant left in a blood matrix on nonporous surfaces in conditions representative of the West African context (28 °C, 90% RH).[9] Factors affecting the persistence of the Ebola virus on surfaces include the type of surface, with shorter survival on porous surfaces compared to nonporous surfaces;[23] the matrix, with longer persistence in blood compared to vomit and no survival in feces;[9] and temperature and relative humidity.[9,24]

The only studies we identified that evaluate the resistance of the Ebola virus to surface disinfection were published by Cook et al.[23,25] Following the ASTM International Standard protocol,[26] they found that a 5 min exposure to 0.5% sodium hypochlorite was sufficient to bring the Ebola virus from a concentration of 4.0 × 10^6 median tissue culture infectious dose ($TCID_{50}$) units/mL to undetectable levels. They recommended using 0.5% or 1.0% sodium hypochlorite, or 67% ethanol, for

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surface disinfection. However, the use of 1 cm discs, as recommended in the ASTM International Standard,[26] is not representative of environmental conditions. Additionally, commonly recommended disinfection practices, such as wiping or covering spills before disinfection, cannot be tested with this protocol.

In the 2014 West African Ebola outbreak, recommendations on disinfecting surfaces and cleaning uncontrolled spills were provided by the World Health Organization (WHO), the Centers for Disease Control and Prevention (CDC), and Doctors Without Borders (MSF) (Table 1). While these guidelines are generally consistent in that they recommend disinfection of surfaces (and other "non-living things") with 0.5% chlorine solutions in ETUs, they differ between agencies in terms of exposure time and recommended practices (e.g., whether or not to pre-clean surfaces and whether or not to cover uncontrolled spills to prevent splashes).

Table 1. Recommendations on Surfaces Disinfection in Ebola Outbreaks

target	action	disinfectant	exposure time	source
hospital and ETU	pre-clean surface	0.5% chlorine	10 min	WHO[19]
household	cover spills	0.5% chlorine	15 min	CDC[40]
hospital	pre-clean surface	"chemical disinfectant for non-enveloped viruses"	not specified	CDC[20]
ETU	do nothing	0.5% chlorine	15 min	MSF[18]

Additionally, the chlorine types used for surface disinfection vary. A total of four types of chlorine were available during the 2014 Ebola outbreak: high-test calcium hypochlorite (HTH), sodium dichloroisocyanurate (NaDCC), and sodium hypochlorite (NaOCl), which can be produced either industrially and pH-stabilized or on-site, using an electrolytic generator. These chlorine sources have varying chemical properties and different advantages and drawbacks (Table 2). For example, low-pH chlorine solutions are expected to be more efficacious for disinfection than high-pH solutions,[27] and NaDCC is expected to be more efficacious than other chlorine types in the presence of organic matter.[28,29] MSF has switched from using both HTH and NaDCC to using only the latter for safety reasons. The current surface disinfection guidelines do not recommend a specific chlorine type (Table 1).

Because of the sporadic occurrence and limited scale of Ebola outbreaks before 2014, there have been few opportunities to study the Ebola virus. In addition, testing using the Ebola virus is expensive and labor-intensive and can only be performed in Biosafety Level 4 facilities. While the persistence on surfaces and resistance to sodium hypochlorite of the Ebola virus was evaluated by Cook et al. in two studies,[23,25] we know of no evidence (1) determining the efficacy of the four chlorine solutions for surface disinfection; (2) documenting the influence of wiping or covering spills on disinfection efficacy; and (3) confirming that a 10 to 15 min exposure to 0.5% chlorine is sufficient to ensure safe disinfection in the Ebola context, in which the virus would typically be shed within a matrix of human waste.

The goal of this research was to provide data for the development of evidence-based recommendations for surfaces disinfection in Ebola outbreaks and other emergency situations by (1) comparing the efficacy of HTH, NaDCC, and stabilized and nonstabilized NaOCl solutions for the disinfection of three Ebola relevant surface types; (2) evaluating how recommended practices such as pre-cleaning or covering spills affect surface disinfection efficacy; and (3) determining how presence of a soil load affects surface disinfection efficacy.

Table 2. Chlorine Types Commonly Used in Emergency Contexts

chlorine type	expected pH	form	advantages	drawbacks
sodium dichloroisocyanurate (NaDCC)	6	granules	easy to ship	smell
			long shelf life	
			does not clog pipes	
high-test hypochlorite (HTH)	11	granules	easy to ship	explosive
			long shelf life	
			does not clog pipes	clogs pipes
stabilized sodium hypochlorite	11	liquid	can be local	shorter shelf life
			does not clog pipes	difficult to ship
nonstabilized sodium hypochlorite	9–11	liquid	can be on-site	shorter shelf life
			does not clog pipes	difficult to ship
				quality control?

MATERIALS AND METHODS

To complete this work, we first selected test organisms and surfaces and then reviewed existing recommendations to design the testing matrix. We then prepared materials, including test organisms, chlorine solutions, surface carriers, and soil load; completed testing in the Environmental Sustainability Laboratory (ESL) at Tufts University (Medford, MA); and analyzed the results.

Development of Testing Matrix. We communicated with MSF water, sanitation, and hygiene staff responsible for setting up and maintaining safety in ETUs about common surfaces in ETUs. Based on this information, we selected three surfaces that were relevant for Ebola contexts: stainless steel (representing medical instruments, including needles) , nitrile (for plastic sheeting and personal protective equipment), and heavy-duty tarp (most likely to be found on floors or walls).

Guidelines from MSF, WHO, and CDC were consistent in recommending the use of 0.5% chlorine for surface disinfection but differed in terms of exposure time and practices (Table 1). We therefore started by testing 0.5% chlorine at the shortest exposure time (10 min) with four recommendations that represented all possible combinations of practices as described in the guidelines: (a) do nothing before applying the chlorine, (b) wipe the disc with a surgical towel, (c) cover the spill with a surgical towel, and (d) wipe the disc and then cover the spill.

Testing all the conditions we had interest in evaluating with the actual Ebola virus was not realistic considering the risks associated with relatively large-scale testing, costs, and the fact that our laboratory is certified at Biosafety Level 2 only. We therefore selected two test organisms: *Escherichia coli* (ATCC

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25922) and bacteriophage Phi6 (HER #102). *E. coli* is a well-established fecal indicator. We used it to test our protocol with a known, robust test organism but do not consider that it would be an appropriate surrogate for the Ebola virus. Phi6 was selected as a surrogate for the Ebola virus after replicating Cook et al.'s[23] experiment testing inactivation of the Ebola virus by 0.5% sodium hypochlorite on 1 cm stainless steel discs with the four bacteriophages MS2 (ATCC 15597-B1), M13 (ATCC 15669-B1), Phi6, and PR772 (HER no. 221). MS2 and M13 were clearly more resistant to chlorine than the Ebola virus and would provide overly conservative results if used as surrogates. PR772, in contrast, was inactivated too quickly to be an appropriate surrogate. Phi6 was slightly more resistant than the Ebola virus, and we therefore considered that it would be an appropriate surrogate for the Ebola virus for the evaluation of surface disinfection efficacy.[30]

The initial testing matrix thus included two test organisms, with and without soil load, on three surfaces with HTH, NaDCC, and stabilized and generated NaOCl following four different procedures for disinfection (Figure 1). This plan called for a total of 192 tests in triplicate for a total of 576 samples.

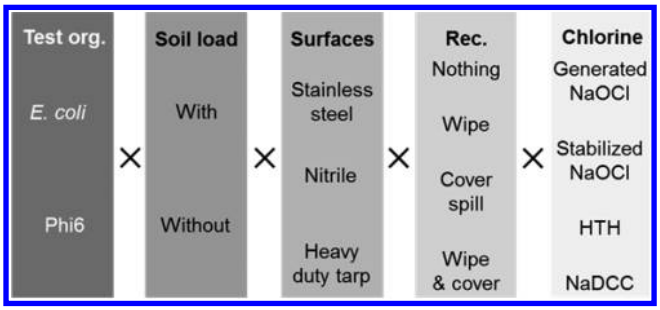

Figure 1. Testing matrix.

We then carried out additional testing to (1) evaluate the effect of increasing the exposure time to 15 min for the conditions in which the test organisms were detected after disinfection and (2) determine whether covering the spill with a surgical towel presoaked in 0.5% chlorine solution would increase disinfection efficacy against *E. coli* on heavy-duty tarp compared to using a dry towel and pouring the 0.5% chlorine solution on top of it.

Preparation of Test Organisms. *E. coli* was streaked onto Luria–Bertani (LB) agar plates that were incubated at 35 °C overnight and then stored at 4 °C. On the night prior to each test day, a single colony was used to inoculate 20 mL of LB broth and incubated at 35 °C with shaking overnight. The culture was then diluted (1:20) and incubated at 35 °C with shaking for approximately 2.5 h or until reaching a density of $\sim 10^{10}$ cells/mL. Lastly, the culture was mixed with either a 0.9% sodium chloride (NaCl) solution or soil load, as described below, for use in the surface disinfection efficacy test.

Phi6 was propagated in *Pseudomonas syringae* (HER no. 1102) by the double agar overlay method:[31] 100 μL of overnight host culture and 100 μL of phage suspension were added to 6 mL of melted NBY soft agar (0.3% agar) kept at 48 °C. The soft agar was then poured onto prepared NBY agar plates (1.5% agar), and the plates were incubated at 26 °C. On the following day, 5 mL of dilution buffer was applied on top of the soft agar, and the plates were left at room temperature for 4 h to let the phages diffuse into the liquid phase, as described in Rossi.[32] The Phi6-containing buffer was then recovered, filtered at 0.45 μm, and stored at 4 °C. On each testing day, the Phi6 suspension was mixed with either dilution buffer or soil load, as described below, for use in the surface disinfection efficacy test.

Chlorine Solutions. NaDCC solution was prepared using Klorsept granules (previously Aquatabs) with 50% active chlorine (Medentech, Wexford, Ireland). HTH solution was produced from commercially available granular calcium hypochlorite with 65% available chlorine (Acros Organics, NJ). Two NaOCl solutions were produced by (1) using laboratory-grade NaCl and an Aquachlor on-site sodium hypochlorite generator (International Equipment & Systems, Inc.; Miami, FL) and (2) diluting a 5.25% laboratory-grade pH-stabilized bleach stock solution (Valtech, Zellenople, PA). Milli-Q water was used for mixing all solutions. On each test day, the concentration of each of the four chlorine solutions was confirmed to be within 10% of a target 0.5% solution (5000 mg Cl/L) by iodometric titration (method no. 8209, Hach Company, Loveland, CO). Disinfection was carried out within 3 h following titration.

Surface Carriers. The surface carriers were 8 cm diameter discs of type 430 brushed stainless steel (McMaster Carr, IL), heavy-duty tarp (Amazon.com), and nitrile (Amazon.com). Before each test, stainless steel discs were sterilized by autoclaving, and heavy-duty tarp and nitrile discs were soaked in 70% ethanol, rinsed with sterile water, and dried using a sterile surgical towel.

Soil Load. A soil load containing 7.80 mg/mL bovine serum albumin (Sigma-Aldrich, St. Louis, MO), 2.52 mg/mL bovine mucin (Alfa Aesar, Ward Hill, MA), and 10.92 mg/mL tryptone (FisherScientific, Fairlawn, NJ) were prepared according to the ASTM International Quantitative Carrier Testing standard.[33]

Testing. To evaluate disinfection efficacy, the 8 cm surface carriers were placed in Petri dishes, and 2 mL of a mixture of 1.36 mL of test organism suspension and 0.64 mL of either soil load or dilution buffer was applied to each surface carrier to mimic an uncontrolled "spill". This spill was left to dry for 1 h in a biosafety cabinet. After drying, one of four recommendations was carried out: (1) no pre-cleaning or covering; (2) "pre-cleaning," consisting of wiping the disc twice in opposite directions with a dry surgical towel before applying chlorine; (3) "covering", in which a dry surgical towel was placed on top of the spill before applying the disinfectant; and (4) both pre-cleaning and covering, in which the same towel was used for pre-cleaning and for covering the spill before applying chlorine. After the recommendation was carried out, 18 mL of one of four 0.5% chlorine solutions was applied and left for 10 min. At the end of the exposure time, chlorine was neutralized by sodium thiosulfate. For *E. coli* testing, sterile tweezers were used to place the disc in a WhirlPak bag (Nasco, WI) containing 300 mL of 0.17% sodium thiosulfate solution and stored on ice; then, 100 mL of sample were used for membrane filtration. For the discs inoculated with Phi6, chlorine was neutralized with 20 mL of 2.55% sodium thiosulfate solution poured on top of the chlorine solution contained in the Petri dish. A 1 mL sample was collected by pipetting and stored in microcentrifuge tubes at 4 °C; next, 100 μL were used for titration. The procedure was different for *E. coli* and Phi6 due to different titration methods. While 100 mL can be processed through membrane filtration, only 100 μL of the sample is used for the Phi6 plaque assay. Using a more concentrated sodium thiosulfate solution for Phi6 resulted in a smaller sample volume and thus lowered the detection limit.

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For each series of tests (24 discs with one test organism, on one surface type, using four chlorine types and following two different disinfection procedures, in triplicate), there was one negative control (in which the spill consisted of 2 mL of either dilution buffer or soil load only) and triplicate positive controls (in which no disinfection was carried out) to evaluate the recovery rate and starting concentration. Samples were tested within 5 h of collection by membrane filtration for *E. coli* using mColiBlue medium (Hach Company, Loveland, CO) and by plaque assays on double layer agar plates for Phi6, following the same procedure as described previously for the propagation.

Data Analysis. Data were initially entered and analyzed in Microsoft Excel 2016 (Redmond, WA), including the calculation of average concentrations, log removals, remaining infective units per surface area, standard errors, and generation of graphs. Statistical tests were then performed using STATA 14.1 (StataCorp LP, College Station, TX).

For all calculations, as the detection limit was 3 colony-forming units (CFU)/100 mL for *E. coli*, samples with zero counts were assigned a value of 1.5 CFU/100 mL. Likewise, for Phi6, the detection limit was 10 plaque-forming units (PFU)/mL, and zero counts were replaced by 5 PFU/mL. A value of 250 CFU or PFU was set for plates that were too numerous to count (TNTC). While this might underestimate the number of recovered organisms, the impact in our case was limited due to the low number of TNTC samples. Because units per unit liquid volume are meaningless for the evaluation of surface disinfection, the results were expressed as the number of infective organism per surface area in figures. The corresponding theoretical detection limits were <1 CFU/cm^2 for *E. coli* and 8 PFU/cm^2 for Phi6.

We evaluated disinfection efficacy in terms of reduction in transmission risk and therefore expressed residual concentration as the number of infective particles per unit area in addition to providing log removals. Studies suggest that 1 to 10% of viruses of viruses can be transmitted from stainless steel to hands after 1 h of drying.[34,35] Considering that 10% of the Ebola virus could be transmitted from a contaminated surface to an individual's hands, the residual virus concentration after disinfection should be less than ten times the infectious dose per "contact spot" (defined as 5 cm^2).[12] Using an intermediate estimate of 5 PFU for the infectious dose, which is thought to be 1−10 PFU,[36] we thus determined that 50 PFU per contact spot or 10 PFU/cm^2 would be an acceptable residual concentration after disinfection. Please note that we used 8 cm in diameter discs, which have a total area of 50 cm^2. We used both log removal and residual PFU/cm^2 in our analysis.

Statistical tests were performed using STATA 14.1 (StataCorp LP, College Station, TX). First, Kruskal−Wallis tests (the nonparametric equivalent to ANOVA) were used to determine if there were differences between the four chlorine types in all the testing, i.e., with or without soil load, for each surface type, and with each pre-cleaning recommendation. In this analysis, triplicate samples obtained for the four chlorine types were compared. For all conditions with no significant difference between chlorine types, data were aggregated, thus creating one group of 12 samples (four chlorine types multiplied by three triplicates) for each recommendation. Then, Kruskal−Wallis tests were performed again to compare the efficacy of recommendations. For the conditions in which significant differences were detected between chlorine solutions, a posthoc analysis was conducted using Dunn's test.

Wilcoxon-Mann−Whitney (WMW) tests were used to determine whether increasing the exposure time or soaking the surgical towel made a significant difference compared to the conditions tested previously (10 min exposure to chlorine and using a dry towel, respectively).

RESULTS

***E. coli* Results.** The surface carriers were inoculated with 3 × 10^{10} CFU on average (range 1.0 × 10^{10} to 1.1 × 10^{11} CFU). The recovery rates, as estimated based on the positive controls after 1 h of drying, were on the order of 10% for nitrile and stainless steel and 20% for heavy-duty tarp.

All tests achieved at least 6 log *E. coli* removal except for one test using wiping only with soil load on heavy-duty tarp, which achieved an average of 5.9 log removal (Table 3). On nitrile and on stainless steel, all recommendations and all chlorine types appear equally efficacious at removing *E. coli*, whereas relative drops in efficacy occurred mainly when covering the spill on heavy-duty tarp (Figure 2).

Table 3. Observed Log Removals and Standard Deviations

surface type	soil load	action	*E. coli* log removal	STD (*E. coli*)	Phi6 log removal	STD (Phi6)
stainless steel	without	nothing	>6.6	0.0	4.1	0.0
		wipe	>6.6	0.0	4.1	0.0
		cover	6.0	0.4	3.4	0.0
		wipe and cover	6.0	0.3	3.4	0.0
	with	nothing	>6.8	0.0	5.7	0.0
		wipe	>6.8	0.0	5.7	0.0
		cover	>7.3	0.0	5.5	0.0
		wipe and cover	7.2	0.2	5.5	0.0
nitrile	without	nothing	6.4	0.0	4.8	0.0
		wipe	>6.4	0.0	4.8	0.0
		cover	6.4	0.2	3.1	0.0
		wipe and cover	>6.5	0.0	3.1	0.0
	with	nothing	>6.9	0.0	3.6	0.0
		wipe	6.8	0.2	3.3	0.4
		cover	>6.8	0.0	5.5	0.0
		wipe and cover	6.7	0.4	5.5	0.0
heavy-duty tarp	without	nothing	6.7	0.8	3.9	0.1
		wipe	6.7	0.9	3.8	0.0
		cover	6.3	0.9	3.4	0.0
		wipe and cover	6.1	1.0	3.4	0.0
	with	nothing	6.0	1.1	5.4	0.0
		wipe	5.9	1.0	3.2	0.0
		cover	6.4	0.9	5.5	0.0
		wipe and cover	6.3	0.9	5.5	0.0

E. coli was detected in 51 out of 288 samples, including 5 times on stainless steel, 40 times on heavy duty tarp, and 6 times on nitrile. The residual contamination in the positive samples ranged from 1 CFU (or 0.06 CFU/cm^2) to TNTC (more than 15 CFU/cm^2).

Based on the Kruskal−Wallis test results (Figure 3), there was no significant difference between chlorine types ($p > 0.05$) except on heavy-duty tarp in the presence of soil load with no

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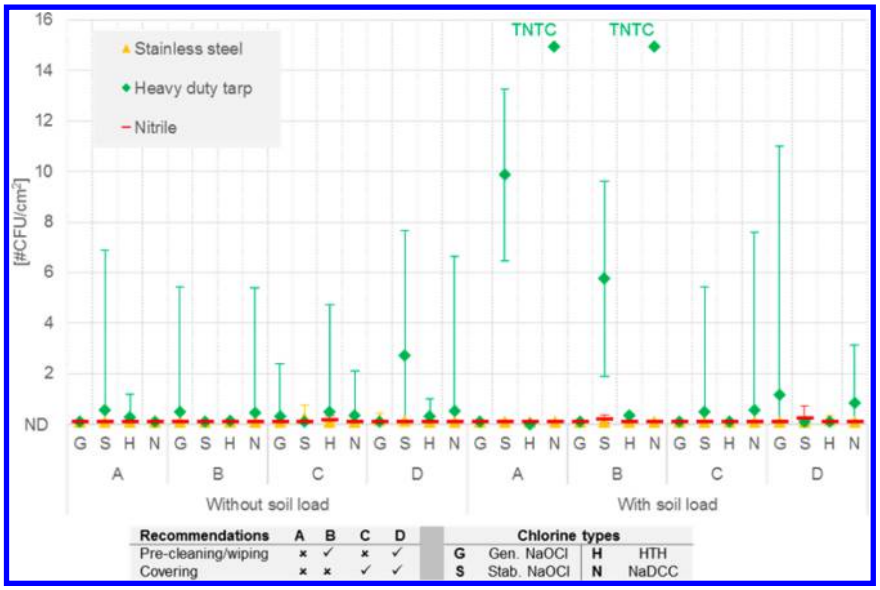

Figure 2. Number of CFU *E. coli* per unit area detected after disinfection. Points correspond to the geometric mean of triplicate samples. Error bars represent standard errors of the mean. "ND" stands for "not detected", and the theoretical detection limit was <1 CFU/cm^2. The points indicated as too numerous to count (TNTC) correspond to cases in which all triplicates were TNTC after disinfection.

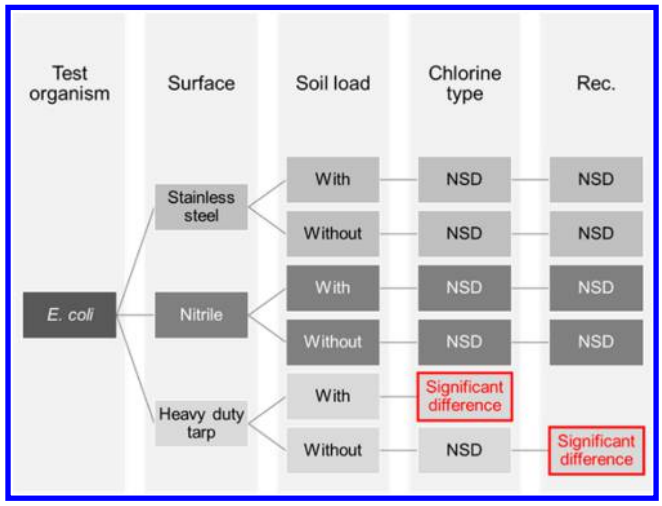

Figure 3. Statistical results of *E. coli* testing. NSD stands for "no significant difference" based on the Kruskal–Wallis test ($p < 0.05$).

pretreatment and with pre-cleaning ($p = 0.02$ in both cases). For the conditions in which all chlorine types appeared equally efficacious, there was no significant difference between recommendations ($p > 0.05$) except on heavy-duty tarp with soil load ($p = 0.03$).

A posthoc analysis was conducted using Dunn's test to compare chlorine types and recommendations, respectively, for the two cases in which statistically significant differences were detected but no chlorine type or recommendation appeared to systematically perform better than the others (results not shown). Only NaDCC was used to evaluate the effect of a 15 min exposure time and of a towel soaked in chlorine solution because significant differences between chlorine type were not routinely identified. On heavy-duty tarp, increasing the exposure time from 10 to 15 min did not significantly increase efficacy in any of the four recommendations with and without soil load, the only exception being in the presence of soil load when nothing was done before applying chlorine (WMW test, $p = 0.03$). Applying a towel soaked in 0.5% NaDCC solution on the spill was not significantly more efficacious than using a dry towel to cover the spill on heavy-duty tarp (WMW test; $p > 0.05$, both with and without soil load).

Phi6 Results. The surface carriers were inoculated with 8×10^7 CFU on average (range 1.3×10^6 to 2.3×10^8 CFU). The recovery rates, as estimated based on the positive controls after 1 h of drying, were on the order of 20% on all three surfaces.

All recommendations achieved at least 3 log Phi6 removal (Table 3). Out of a total of 288 samples, Phi6 was detected after disinfection in six samples on nitrile and in no samples on stainless steel or heavy-duty tarp. The residual contamination in the positive samples ranged from 16 to 80 PFU/cm^2 after disinfection. Due to the limited number of positive samples, statistical tests were not conducted on Phi6 results. The geometric mean of triplicates exceeded the target of 10 residual PFU/cm^2 in two instances (Figure 4): Phi6 was detected in the presence of soil load when nitrile discs were pre-cleaned with both generated and stabilized NaOCl. For the two conditions in which Phi6 was detected on nitrile after a 10 min exposure to 0.5% chlorine, increasing the exposure time to 15 min led to all nondetectable levels.

DISCUSSION

We conducted a systematic evaluation of the efficacy of surface disinfection recommendations for Ebola contexts, including testing two organisms, two soil load conditions, four surface cleaning recommendations, and four chlorine types on three surfaces commonly used in ETUs. Results indicated that test organism, surface type, and covering spills influenced surface disinfection efficacy, while chlorine type, soil load, and pre-cleaning did not. Overall, the recommendations provided during the 2014 EVD outbreak for surface disinfection appeared to be efficacious at removing *E. coli* and Phi6 from representative surfaces. Based on our results, we recommend a 15 min exposure time to 0.5% chlorine to ensure safe surface disinfection in EVD contexts. These results (1) are consistent with previous results while also providing further context-specific evidence, (2) answer questions about chlorine type while raising questions about the role of soil load and how to

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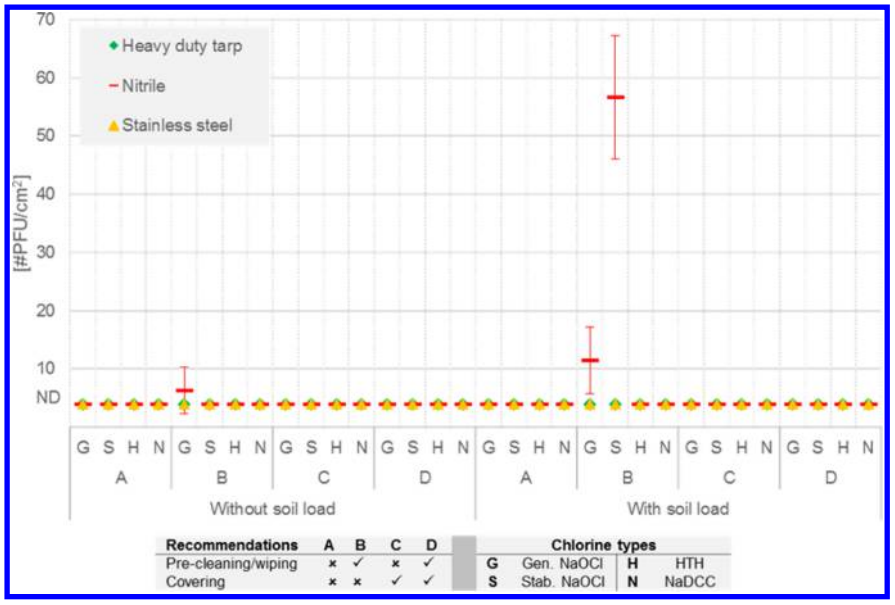

Figure 4. Number of Phi6 PFU per unit area detected after disinfection. Points correspond to the geometric mean of triplicate samples. Error bars represent standard errors of the mean. "ND" stands for "not detected", and the theoretical detection limit was 8 PFU/cm^2.

appropriately model soil load in these contexts, (3) highlight the important role of test organism and surface type in disinfection, and (4) inform how to conduct research on surface disinfection efficacy in further outbreak situations.

The Cook et al.[23,25] studies documented a 5 min contact time with 0.5% sodium hypochlorite to completely disinfect stainless steel surfaces inoculated with the Ebola virus. Our data expand on their work as we found that 10 min were sufficient to disinfect stainless steel using Phi6 as an Ebola surrogate, but additional contact time was needed specifically on nitrile to ensure full disinfection under all tested conditions. This is also consistent with a previous study in which environmental sampling was conducted in an isolation ward in Uganda in 2000. Bausch et al.[13] found that CDC and WHO recommendations were efficacious, as Ebola was not isolated from surfaces after routine disinfection. However, the recommendations were more conservative during that time, as they recommended a 1% chlorine solution with a 15 min contact time and that a 10% chlorine solution should be used for heavy or dense spills.[37] A more recent study conducted in Sierra Leone in 2015 suggests that environmental decontamination using 0.5% sodium hypochlorite was mostly effective at removing Ebola RNA from surfaces around the bedside, where contamination was detected most often.[11] Our results also indicate 0.5% chlorine solutions should be efficacious.

In contrast to beliefs that some chlorine types may be more efficacious than others, we found all chlorine types appeared equally efficacious for the disinfection of surfaces against *E. coli* and Phi6. We attribute this to the high chlorine dose (0.5%) applied. As such, we recommend using whichever chlorine source is available, safe to handle, and maintains an appropriate concentration.[38]

The presence of soil load did not affect disinfection efficacy at a chlorine concentration of 0.5%. We used the soil-load mixture recommended in the ASTM International Standard,[26] and the chlorine demand of the spill with soil load represented less than 0.01% of the amount of applied chlorine. Again, we hypothesize that soil load did not impact efficacy because of the high chlorine concentration applied. While this is likely representative of field practices, in which chlorine will be generously applied to ensure disinfection, further research is needed to develop new matrices more representative of liquid Ebola bodily wastes and their impact on disinfection efficacy.

Our results suggested that test organism and surface type do play an important role in determining disinfection efficacy. We found that *E. coli* was more challenging to disinfect on heavy-duty tarp, and Phi6 was more challenging to disinfect on nitrile, suggesting that different mechanisms are responsible for disinfection of the two organisms. A literature search provided little information on which fundamental mechanisms might explain the observed difference. We hypothesize that physical properties of the surface (such as roughness) as well as surface charge (of both the surface and the test organism) impacted disinfection efficacy. The fact that increasing the exposure time from 10 to 15 min did not improve disinfection efficacy against *E. coli* on heavy-duty tarp suggests that bacteria did not enter into contact with the disinfectant, possibly due to some physical protection provided by the roughness of heavy-duty tarp. It is unclear why such an effect would not be observed with Phi6, and additional research is indicated to investigate our hypotheses. The widespread use of both heavy-duty tarp and nitrile in emergency settings makes these results of particular concern. It is recommended to conduct future research with other surfaces commonly found in ETU settings, including paper, cardboard, cotton, rubber, latex, and concrete.

The international recommendations for surface disinfection in Ebola contexts are particularly different in terms of practices. It has been suggested to wipe and to cover spills, and recently, to pre-clean surfaces with water and detergent.[39] While pre-cleaning reduces the volume of spill to be disinfected, such a practice generates contaminated waste and can result in exposure of healthcare personnel to infectious materials. Although we were not able to test for the multiplicity of pre-cleaning practices that are likely to be encountered in the field (with or without soap, with dry and wet towels, and accounting for individual variability), our results suggest that wiping the surface before applying chlorine is unlikely to increase disinfection efficacy. This is consistent with a study of surface

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disinfection involving several viruses, in which Tuladhar et al.[12] found that inactivation by a 0.1% chlorine solution was proportionally more important than the action of wiping. Covering spills is recommended to avoid disease transmission by splashes,[18] and our results with Phi6 suggest that it does not affect disinfection efficacy. We recommend covering spills with a cloth soaked in 0.5% chlorine and leaving it for 15 min if disease transmission via splashes is a concern, as is the case with EVD due to the very low infectious dose.

Our study had limitations, including that (1) the comparison of *E. coli* and Phi6 is limited by different starting concentrations and detection limits; (2) although the recovery rates were similar for both organisms, we cannot rule out that some of the observed differences could be due to variability in recovery; (3) we used a surrogate organism instead of the actual virus, albeit one selected based on a comparison between the surrogate (tested in our laboratory) and Ebola virus (tested by Cook et al.[23] in a Biosafety Level 4 laboratory); (4) we believe that our protocol, using 8 cm in diameter discs instead of the 1 cm diameter surface carriers recommended in the ASTM Standard,[33] simulates field conditions more accurately despite the fact that 2 mL spills are still unlikely to reflect the extent of environmental contamination experienced in ETUs; (5) our standardization of pre-cleaning recommendations does not account for further variability in pre-cleaning practices applied in ETU settings; (6) the ASTM soil load might not be representative of the matrices in which the Ebola virus would be shed by patients; and (7) temperature and relative humidity in the laboratory were both lower and more controlled than those commonly found in ETU settings.

Despite these limitations, we feel our data contribute to the understanding of, and assist in providing an evidence base for, surface disinfection practices in outbreaks. In particular, our results support the recommendation of a 15 min exposure to 0.5% chlorine (independent of chlorine type, surface type, practices, and presence of organic matter) as an efficacious measure to interrupt EVD transmission via fomites. Using Phi6 as a surrogate allowed us to carry out extensive testing and to identify critical conditions; we recommend evaluating the resistance of the Ebola virus (at Biosafety Level 4) to a 15 min exposure to 0.5% chlorine, without pre-cleaning or covering, with or without soil load, on nitrile and, if possible, on other surfaces, to confirm the results presented here.

Further research is required to investigate how surface properties and complex matrices affect disinfection efficacy as well as to understand the role of wiping or covering spills. A more-fundamental understanding of the mechanisms affecting disinfection efficacy and of the physicochemical interactions between microorganisms and surfaces will allow extension of these results to different pathogens with more flexibility.

Our study was carried out in response to the 2014 Ebola outbreak and focused on evaluating the efficacy of existing recommendations in place during that outbreak. It is recommended that future research be completed to develop appropriate recommendations, not just to test the efficacy of existing recommendations. For instance, using NaDCC granules instead of a liquid chlorine solution to cover uncontrolled spills has been identified as an efficacious procedure against bacteria in developed country hospitals.[29] Additional research is needed to evaluate the adequacy of this, and other novel, protocols as a possible intervention in ETU contexts.

For diseases with infectious doses as low as EVD, multibarrier approaches (including personal protective equipment, handwashing, disinfection of surfaces, and safe waste management) should be used to minimize transmission risk. However, multibarrier approaches (particularly those with waiting times) are burdensome in the ETU context due to the maximum time responders can spend in PPE. A broader discussion around the best way to minimize transmission risk and the appropriate number and level of barriers to use in healthcare facilities and communities facing situations such as EVD is necessary. For example, it has been suggested to depend on PPE primarily and not be concerned with disinfecting spills in the ETU context. Ideally, further discussion will involve responders, members of the scientific community, and international organizations to address the appropriateness of existing recommendations and how they can be adjusted to increase preparedness for future outbreaks.

AUTHOR INFORMATION

Corresponding Author

*Phone: 1 617 483 2045; e-mail: karin.gallandat@tufts.edu.

ORCID

Karin Gallandat: 0000-0001-6412-1483

Notes

The authors declare no competing financial interest.

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