Laboratory efficacy of surface disinfection using chlorine against *Vibrio cholerae*

Gabrielle M. String, Eduardo Vargas Gutiérrez and Daniele S. Lantagne

ABSTRACT

Disinfecting surfaces with chlorine is commonly conducted in cholera outbreaks to prevent ongoing fomite-based transmission, yet evidence gaps have led to contradictory guidance. In this study, we tested the efficacy of spraying and wiping chlorine on five representatives non-porous and five porous surfaces to remove *Vibrio cholerae*. In total, 120 disinfection tests were run in replicate on carriers inoculated with 1.02×10^7–1.73×10^8 *V. cholerae* CFU/cm^2. Surfaces disinfected by spraying 0.2% chlorine had >3 log reduction value (LRV) on 7/10 and 9/10 surfaces at 1 and 10 min, respectively; and 2.0% chlorine on 9/10 and 10/10 surfaces at 1 and 10 min, respectively. Surfaces disinfected by wiping 0.2% chlorine had >3 LRV on 3/10 and 7/10 surfaces at 1 and 10 min, respectively; and 2.0% chlorine on 8/10 surfaces at 1 and 10 min. We found no significant differences between chlorine types ($p < 0.05$), higher reductions with spraying compared to wiping ($p = 0.001$), and lower reductions on porous compared to non-porous surfaces ($p = 0.006$ spraying and $p < 0.001$ wiping). Our results support using 0.2% chlorine sprayed on all surfaces, or wiped on most non-heavily soiled surfaces, and a 2.0% concentration on contaminated porous surfaces; and emphasize surfaces must be visibly wetted to achieve disinfection.

Key words | chlorine, disinfection, spraying, surface, *Vibrio cholerae*, wiping

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HIGHLIGHTS

- Chlorine disinfection of surfaces in cholera outbreaks is widespread, yet surfaces common to low-resource contexts are under-researched.
- We tested the efficacy of chlorine disinfection to reduce *Vibrio cholerae* on 10 non-porous and porous surfaces.
- Spraying chlorine, and disinfection of non-porous surfaces, had higher *V. cholerae* reductions.
- Results support the use of 0.2% chlorine on most surfaces and 2.0% on contaminated porous surfaces.

INTRODUCTION

Cholera continues to affect vulnerable populations globally, with an estimated 1.3 billion people at risk for cholera in endemic countries and a 2015 estimate of 2.9 million cases annually (Ali *et al.* 2015). Infection via ingestion of the *Vibrio cholerae* (*V. cholerae*) bacterium can cause acute diarrhea and vomiting leading to severe dehydration and shock (Sack *et al.* 2004). In 2019, an estimated 2,485 deaths and 128,121 cases were reported to WHO globally as a result of cholera infection (WHO 2019a). In 2017, the Global Task Force on Cholera Control launched a strategy aimed to reduce cholera deaths by 90% and eliminate cholera in 20 countries by 2030 (WHO 2019b). The ambitious

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plan focuses on early detection and response to contain outbreaks, multisectoral targeted approaches to prevent recurrence, and creation of coordination mechanisms for cholera control (Global Task Force on Cholera Control 2017).

Cholera particularly affects populations lacking access to adequate water, sanitation, and hygiene (WASH), and often occurs in ongoing complex emergencies (Gayer *et al.* 2007; Spiegel *et al.* 2007; Cronin *et al.* 2008; WHO 2019b). Traditionally considered a waterborne disease, evidence suggests that cholera is also transmitted person-to-person and environment-to-person (e.g. food and fomites) (Fung 2014; Richterman *et al.* 2018; Gallandat *et al.* 2020a). Cholera outbreaks are mitigated by strategies involving implementation of both preparedness and response activities, including behavior change communication, distribution of oral cholera vaccines, promotion of water treatment and safe sanitation, and personal and environmental hygiene activities (Lamond & Kinyanjui 2012; ACF 2013; Olson *et al.* 2017). Recently, there has been an increased focus on case-area targeted interventions (CATI) for cholera control, as individuals living within 50 m of a cholera case are 23–56 times as likely to contract cholera as those further away (Finger *et al.* 2018; Ratnayake *et al.* 2020). One commonly implemented CATI is household spraying, where a dedicated team is sent to disinfect the case household, and to some degree neighbor households, by spraying chlorine on household surfaces (Yates *et al.* 2018). Additionally, it is recommended to wipe surfaces with chlorine in healthcare facilities (Olson *et al.* 2017; Centers for Disease Control & Prevention 2018; Dunoyer 2013; UNICEF 2013).

Surface disinfection is critical as many surfaces common to healthcare and household settings can act as fomites, or transmission vectors, for *V. cholerae* bacteria (Sugimoto *et al.* 2014; Gallandat *et al.* 2020a; Kolus *et al.* in press). Additionally, the surface material can impact a pathogen's persistence and resistance to disinfection (Tuladhar *et al.* 2012). Currently, there is a research gap on the efficacy and effectiveness of spraying and wiping chlorine for removal of *V. cholerae*, particularly for surfaces relevant to low-resource contexts. In a recent systematic review, only one study evaluating chlorine-based surface disinfection for *V. cholerae* was identified (Gallandat *et al.* 2020b). The study found 0.62% sodium hypochlorite and acetic acid sprayed on aluminum, glass, and carpet reduced *V. cholerae* >5 log reduction value (LRV), but recovery was too low (1–5%) on wood and concrete to draw conclusions (Calfee & Wendling 2013).

A recent study on household spraying effectiveness in cholera outbreaks found spraying had the potential to remove *V. cholerae* from household surfaces only if they were sprayed until wet with an appropriate concentration of chlorine solution (Gallandat *et al.* 2020a). Additionally, this study noted programmatic challenges involved in implementing spraying consistently, including the fact that only households of cases that reach a treatment center (often 2–6 days after cholera onset) were sprayed; the challenges in correctly identifying a case household in the field; the high resource needs to transport spraying teams; and the context-specific perceptions from recipient households including stigmatization and program refusal.

In the absence of evidence, international and national guidance on disinfection by spraying and wiping chlorine on household and healthcare facilities is mixed and contradictory. Despite the historical ubiquity of household spraying in cholera outbreak response (Taylor *et al.* 2015), a recent scoping review of only international guidelines found that four leading agencies do not recommend disinfection of households by spraying with chlorine and three agencies recommend the distribution of household disinfection kits (D'Mello-Guyett *et al.* 2020). Guidelines indicate either deprioritizing household spraying or explicitly do not recommend spraying because of the following reasons: lack of evidence at reducing disease spread, risk of stigmatizing patients and families, possibility of recontamination of surfaces from asymptomatic or convalescing household members, delay in reaching patient household after cholera onset, high susceptibility of *V. cholerae* to desiccation on surfaces, lack of spraying recommendations from agencies, possibility of damaging household surfaces, and large number of staff and resources required to implement the program (Lamond & Kinyanjui 2012; ACF 2013; Olson *et al.* 2017; UNICEF 2013). However, these same guidelines indicated the use of spraying in healthcare facilities using 0.2% chlorine to disinfect beds, floors, and walls (when a patient is not present), feet/shoes when entering and exiting the facility, and soiled equipment. Furthermore, they recommend a 2.0% chlorine solution to disinfect fecal spills.

Some of these guidelines recommend distribution of a disinfection kit containing materials for households to disinfect their own dwelling by wiping surfaces and washing objects and cloths with a disinfectant such as detergent or 0.5–2% chlorine as a kit can be used multiple times to prevent new cholera cases within the household (ACF 2013; Olson *et al.* 2017; UNICEF 2013).

Thus, the goal of this study was to fill the evidence gap on the efficacy of spraying and wiping chlorine on household and healthcare facility surfaces to remove *V. cholerae*, as mediated by chlorine type and concentration, contact time, application method, and surface material.

MATERIALS AND METHODS

To conduct this study, we designed a test matrix based on international recommendations, made surface carriers and chlorine solutions, carried out the disinfection tests, and analyzed data. All experiments were completed in the Environmental Sustainability Laboratory at Tufts University (Medford, MA, USA).

Test matrix

We selected ten materials for study inclusion that were representative of surfaces found in low-income healthcare facilities and households. Specifically, we tested five non-porous surfaces, including stainless steel, polypropylene plastic, glazed ceramic, nitrile, and heavy-duty tarp, and five porous surfaces, including wood, terracotta, foam, cloth, and packed dirt. We included all three chlorine compounds commonly available in low-resource humanitarian contexts (Wells *et al.* 2016), sodium hypochlorite (NaOCl), high-test hypochlorite (HTH), and sodium dichloroisocyanurate (NaDCC) in our test matrix.

In healthcare facilities, the Centers for Disease Control and Prevention recommends using a 0.05% chlorine solution for handwashing, laundry, and rinsing dishes; a 0.2% solution for footbaths, cleaning floors, bedding, and latrines; and a 2.0% solution for disinfecting vomit, feces, and corpses (Centers for Disease Control & Prevention 2018). Other organizations suggest similar solution preparation for spraying and household disinfection kits (Lamond & Kinyanjui 2012; ACF 2013; Olson *et al.* 2017; UNICEF 2013), and it was observed during an efficacy evaluation that organizations sprayed 0.2% and/or 2.0% chlorine in households (Gallandat *et al.* 2020a). Therefore, we chose to test 0.2% and 2.0% chlorine solutions in our test matrix. Inoculated surfaces were exposed to the chlorine solution for 1 and 10 min, to represent an order of magnitude difference in contact times and approximating behavior in real-world applications. The full test matrix also included both application of the chlorine by spraying and by wiping a pre-soaked towel across the surface (Figure 1).

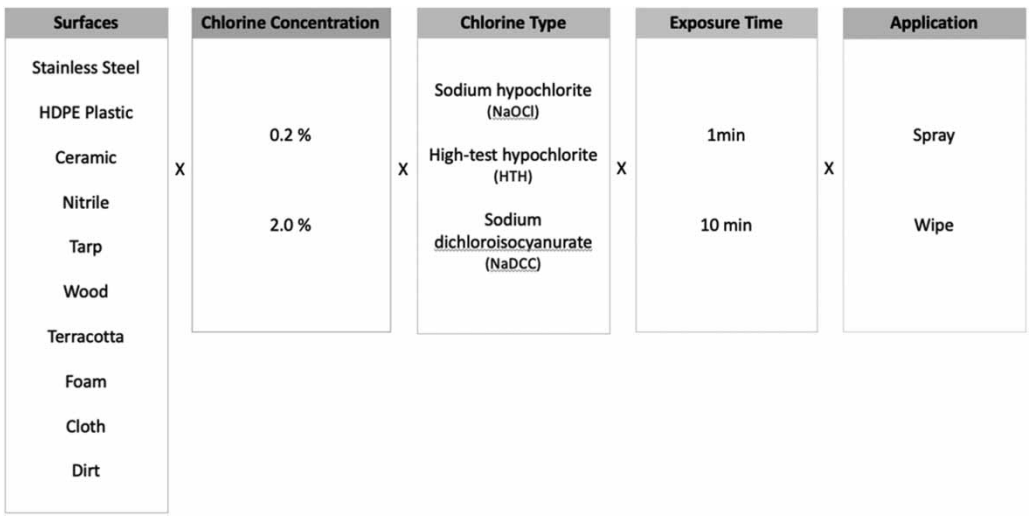

Figure 1 | Test matrix.

Surface carriers

Eight surface carriers were 8 cm in diameter, including 430 brushed stainless steel (McMaster-Carr, IL, USA), polypropylene (McMaster-Carr, IL, USA), nitrile (Amazon.com; Small Parts, WA, USA), heavy-duty tarp (Amazon.com; Direct Tarp, WA, USA), wood (The Home Depot, GA, USA), polyurethane foam (McMaster-Carr, IL, USA), cloth (Amazon.com; Hanesbrands, NC, USA), and packed dirt on stainless steel. The dirt surface carriers were made by combining 8 g of Mystic River (Medford, MA, USA) creekbed sediments, previously sieved through an 18×14 mesh, rinsed, and dried at 100 °C for 72 h in an oven, with 2 g of cactus soil (The Home Depot, GA, USA), and 5 mL of Type 1 laboratory-grade water (Milli-Q® Reference, MilliporeSigma, MA, USA) filtered through a 0.22 μm filter (Millipak®, MilliporeSigma, MA, USA), hereafter termed 'Milli-Q water'. Prior to mixing with Milli-Q water, the sediment and soil were sterilized by autoclaving. The mixture was then aseptically molded onto a stainless steel carrier, placed in a covered Petri dish, and allowed to dry for 18 h prior to use. The two remaining surface carriers were 10 cm in diameter, including the glazed ceramic (The Home Depot, GA, USA) and terracotta (The Home Depot, GA, USA). Before each experimental run, the stainless steel, ceramic, wood, terracotta, foam, and cloth carriers were sterilized by autoclaving. The plastic, nitrile, and tarp carriers were soaked for 18 h in 70% ethanol, rinsed with Milli-Q water, and dried using a sterile towel.

Chlorine solutions

NaOCl solutions were prepared by diluting 5.25% laboratory-grade NaOCl (Austin's A-1 Bleach, PA, USA) with Milli-Q water. HTH solutions were prepared from 65% available chlorine granular calcium hypochlorite (Acros Organics, NJ, USA) dissolved in Milli-Q water. NaDCC solutions were prepared by dissolving 50% active chlorine Klorsept granules (Medentech, Wexford, Ireland) in Milli-Q water. Solutions were stored at room temperature in opaque plastic bottles. Two hours prior to each disinfection test, the concentration of every solution was confirmed to be within 10% of the target concentrations, 0.2% (2,000 mg Cl/L) or 2.0% (20,000 mg Cl/L), using iodometric titration (Method 8209, Hach Company, CO, USA). For spraying tests, chlorine solutions were placed in Qorpak™ spray bottles (Berlin Packaging, IL, USA). For wiping tests, Huck style surgical towels (MSC Industrial Direct, NY, USA) were cut into 5×5 cm squares and autoclaved. The morning of testing, towels was placed in individual labeled sterile Petri dishes and 3 mL of corresponding chlorine solution were pipetted onto each towel.

Disinfection tests and sample processing

V. cholerae (El Tor N16961, ATCC 39315) was streaked onto Tryptic Soy Agar plates, incubated at 35 °C for 24 h, and stored inverted at room temperature in a locked box for 5 days. The night prior to each test, a colony was isolated from the streak plate and used to inoculate 10 mL of Tryptic Soy Broth (TSB) in a sterile Falcon tube, and incubated at 35 °C on a shaking plate for 12–18 h. The culture was then diluted (1:25) in 50 mL of sterile TSB and incubated at 35 °C on a shaking plate for 2.5 h, or until a concentration of $\sim 10^{10}$ cells/mL was reached, as estimated using a spectrophotometer (OD = 600 nm) (GeneQuant 100, Biochrom Division of Harvard Bioscience, MA, USA). The culture was used immediately to inoculate surface carriers.

Disinfection tests were conducted using methods adapted from the ASTM International Quantitative Carrier Test Method as previously published (ASTM E35 Committee n.d.; Gallandat *et al.* 2017). Surface carriers were placed in individual sterile Petri dishes and laid out in a biosafety cabinet at room temperature and approximately 10% relative humidity. Carriers were inoculated by pipetting 2 mL of culture onto the surface in 20 droplets of 100 μL each using a multi-channel pipette and following the same pattern on each carrier. Carriers were left to dry for 1 h at room temperature.

After drying, chlorine was applied to the surface by either spraying five times (resulting in visibly wet surfaces) or a dry surgical towelette soaked in 3 mL of chlorine immediately prior to use was firmly wiped across the surface using constant pressure in an 'x' pattern. After application, the chlorine exposure time was monitored with a stopwatch and either 1 or 10 min was allowed to pass. At the end of the exposure, ethanol sterilized forceps were used to pick up the surface carrier and place it into a prepared 710 mL

Whirl-Pak® bag (Nasco, Fort Atkinson, WI, USA) containing 300 mL of phosphate-buffered saline (PBS, pH ~ 7.5) with sodium thiosulfate to neutralize the chlorine. Remaining liquid in the Petri dish was also poured into the Whirl-Pak® bag to aid recovery. Whirled bags were placed on ice for no more than 2 h until sample processing. All tests were carried out in duplicate, and all testing (chlorine types/concentrations/contact times) for one application method and one surface type were conducted in 1 day. For each contact time, two positive controls and one negative control surface carrier were also tested, where PBS with sodium thiosulfate was applied in place of chlorine. Furthermore, for wiping tests, two additional positive controls were added to ascertain the difference between wiping the surface and wiping the surface with chlorine.

Samples were processed by passing 100 mL of the sample from each Whirl-Pak® bag across a 0.22 μm polycarbonate filter (MilliporeSigma, Burlington, MA, USA) via membrane filtration. The membranes were each placed in a 50 mL Falcon tube containing 12 mL of PBS and vortexed for 5 min to detach *V. cholerae* from the membrane surface and resuspend in the PBS following the protocol from Huq *et al.* (2012). Appropriate serial, tenfold dilutions of each sample were then prepared in 900 μL of PBS in sterile Eppendorf tubes and kept on ice until plating within 2 h. Samples were spread (250 μL) onto thiosulfate-citrate bile salts agar (BD Difco, East Rutherford, NJ, USA), incubated inverted at 35 °C for 24 h, and all colonies counted.

Data analysis

Data were entered and analyzed in Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA). The geometric mean concentrations were calculated for each cultured sample, accounting for dilutions and reported in CFU/cm^2. Plates with zero values were replaced with half of the theoretical detection limit, 0.5, which corresponded to 0.92–1.43 CFU/cm^2 in least dilute samples, depending on the surface carrier area, and plate counts above the detection limit were assigned a value of 250 CFU. LRVs and standard errors were calculated (De Vries & Hamilton 1999); wiping results were calculated as the reduction between wiping with a chlorine soaked towel and wiping with a PBS soaked towel, thereby removing the effect of the wiping action to test the effect of the disinfectant. Additionally, the LRV between positive controls and wiping with a PBS soaked towel was calculated to characterize the effect of the wiping action alone. Percent recoveries on each surface were calculated from log base 10 values. By the chlorine application method, Kruskal–Wallis tests were used on replicate samples to determine if LRVs were different by chlorine type for each surface, chlorine concentration, and contact time tested. Where there was no difference, data were aggregated thereby removing the stratification by chlorine type. Geometric mean LRV and geometric standard deviation were calculated for aggregated samples. Data were analyzed by grouping test combinations that produced ≥4 LRV as compared to those that did not as ≥4 LRV is a common USEPA threshold for disinfectants against resistant bacteria (USEPA OCSPP 2018). For this aggregated data, Pearson's Chi-squared test was used to determine if the application method or surface porosity were associated with ≥4 LRV.

RESULTS

In total, 120 tests were run in duplicate with positive and negative controls. The carriers were inoculated with 1.02×10^7–1.73×10^8 CFU/cm^2 and positive controls ranged 1.04×10^3–2.14×10^7 CFU/cm^2, depending on the surface carrier area. The recovery on porous surfaces including wood and terracotta was 41–84% and the recovery on non-porous surfaces was 71–98%.

Surfaces disinfected by spraying with 0.2% chlorine (all types) had >3 LRV on 7/10 surfaces with a contact time of 1 min and 9/10 surfaces with a contact time of 10 min (Figure 2). Surfaces where >3 LRV for all types of 0.2% sprayed chlorine at 1 min was not achieved included wood, foam, and dirt; at 10 min included dirt. For the same conditions, with a chlorine concentration of 2.0%, 9/10 surfaces had >3 LRV at 1 min contact time; all surfaces had >3 LRV at 10 min (Figure 2).

The mechanical effect of wiping alone was inconsistent and led to a −0.77 to 1.02 LRV on non-porous surfaces and −0.62 to 1.33 LRV on porous surfaces, compared to positive controls. Surfaces disinfected by wiping

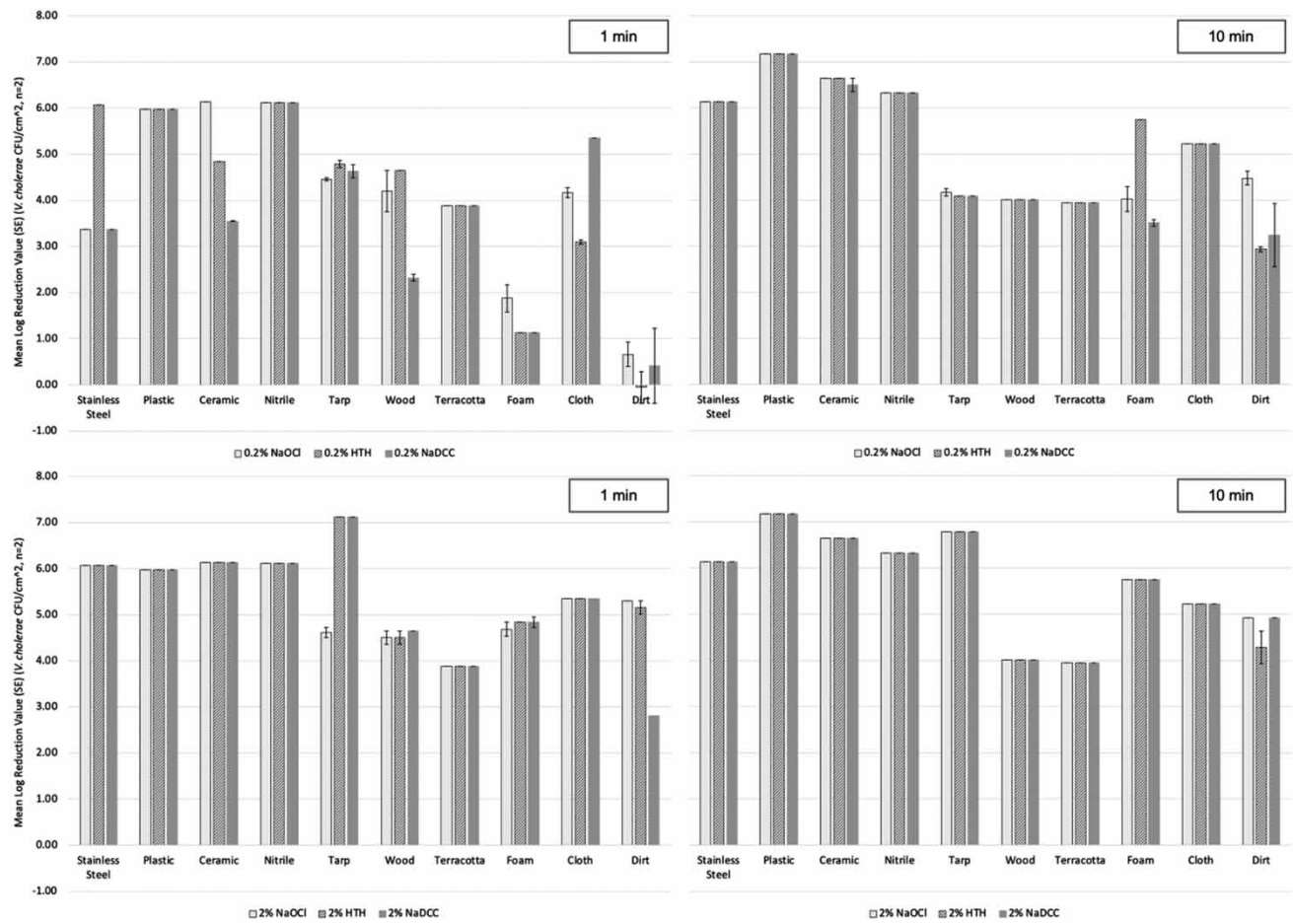

Figure 2 | Mean log reduction (standard error) of *V. cholerae* by spraying. The reduction values [$\log_{10}$ CFU/cm^2] are presented for each surface and chlorine type, stratified by chlorine concentration and contact time. The error bars represent the standard error of the mean of replicates.

with 0.2% chlorine (all types) had >3 LRV on 3/10 surfaces with a contact time of 1 min and 7/10 surfaces with a contact time of 10 min (Figure 3). The only surfaces to achieve >3 LRV when wiped with 0.2% chlorine and exposed for 1 min were stainless steel, glazed ceramic, and nitrile. For the same conditions, with a chlorine concentration of 2.0%, 8/10 surfaces had >3 LRV at both 1 and 10 min contact time (Figure 3). A >3 LRV in *V. cholerae* was not consistently achieved on foam or dirt surfaces when wiped with a chlorine soaked towel.

There was no statistical difference ($p > 0.05$ all test combinations) between chlorine types for spraying and for wiping; therefore, we aggregated data across chlorine types (Figure 4). Spraying or wiping with 0.2% chlorine and 1 min of contact time had significantly lower LRVs than other tested disinfection methods on most surfaces. Reduction of *V. cholerae* was more consistent between concentrations and contact times across surfaces that were sprayed than surfaces that were wiped. Overall, after aggregating by chlorine type, there were 40 tested combinations (surfaces, concentrations, and contact times) each for spraying and wiping. For spraying, 32 test combinations resulted in >4 LRV; for wiping, 17/40 achieved >4 LRV. Spraying chlorine was associated with a higher LRV compared to wiping ($p = 0.001$). When the data are further grouped by non-porous versus porous surfaces, spraying resulted in >4 LRV on all 20 tests conducted on non-porous surfaces and 12/20 tests on porous surfaces. By contrast, when wiped, 15/20 tests on

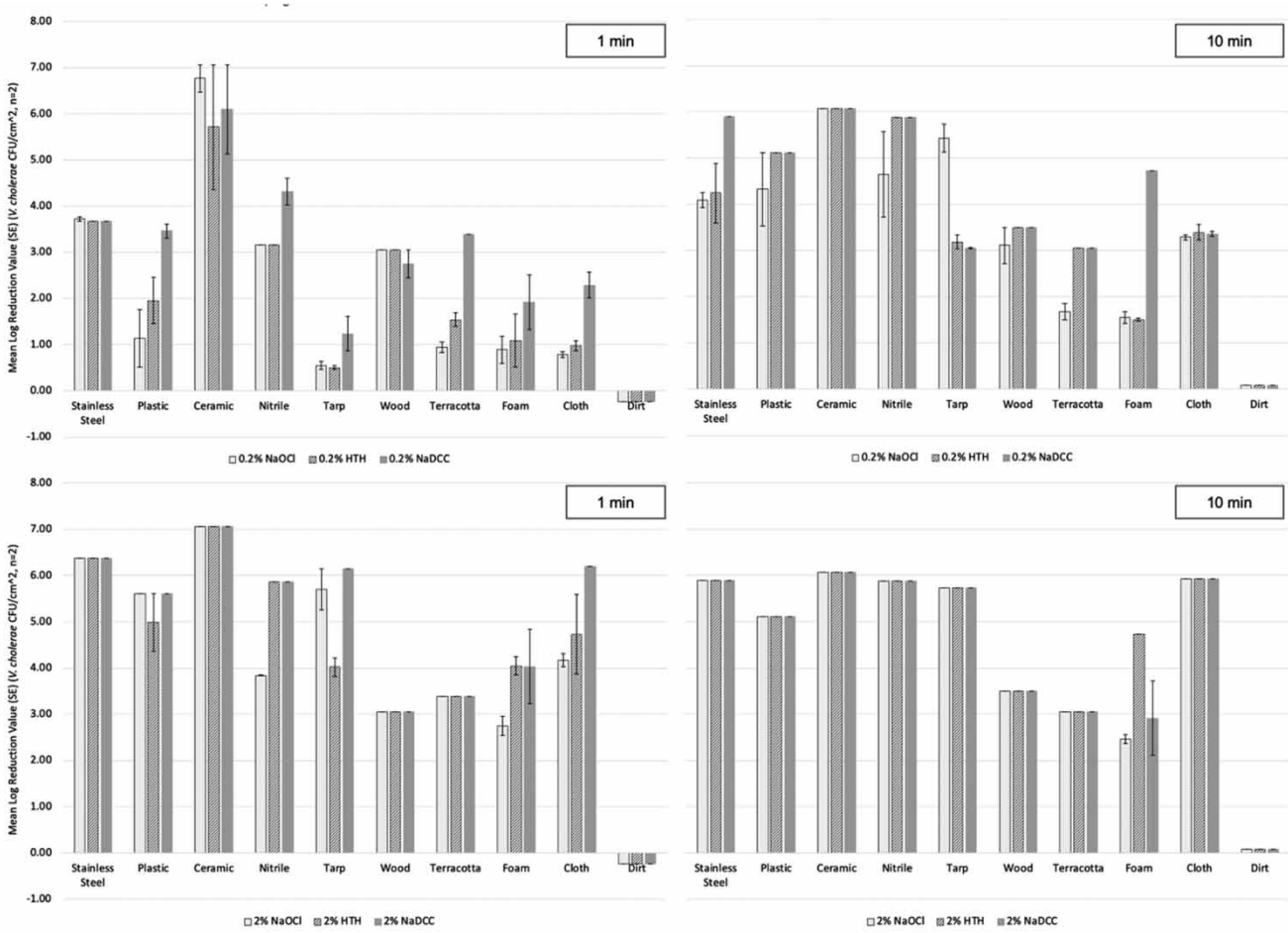

Figure 3 | Mean log reduction (standard error) of *V. cholerae* by wiping. The reduction values [$\log_{10}$ CFU/cm^2] are presented for each surface and chlorine type, stratified by chlorine concentration and contact time. The error bars represent the standard error of the mean of replicates.

non-porous surfaces and 2/20 tests on porous surfaces resulted in >4 LRV. Across both application methods, disinfection of a non-porous surface was associated with a higher LRV than a porous surface ($p = 0.006$ for spraying and $p < 0.001$ for wiping).

DISCUSSION

We tested the efficacy of chlorine disinfection by spraying and wiping *V. cholerae* from five non-porous surfaces and five porous surfaces common to low-resource setting healthcare facilities and households. Included in our testing three different chlorine types were evaluated, two recommended concentrations, and two disinfectant contact times. We found that *V. cholerae* was reduced >3 LRV on most surface types when sprayed or wiped with either 0.2 or 2.0% chlorine and exposed for 10 min. Additionally, we found that the only tested combination to achieve >3 LRV on packed dirt was spraying with 2.0% chlorine and exposing for 10 min. Overall, we have filled a gap in the research and found that spraying and wiping can be efficacious in removing *V. cholerae* from surfaces. In our testing, we found no significant differences between chlorine types, significantly higher log reductions on surfaces that were disinfected by spraying compared to wiping, and significantly lower log reductions on porous surfaces as compared to non-porous ones.

Previous research on surface disinfection efficacy using *Escherichia coli*, a conservative surrogate for culturable

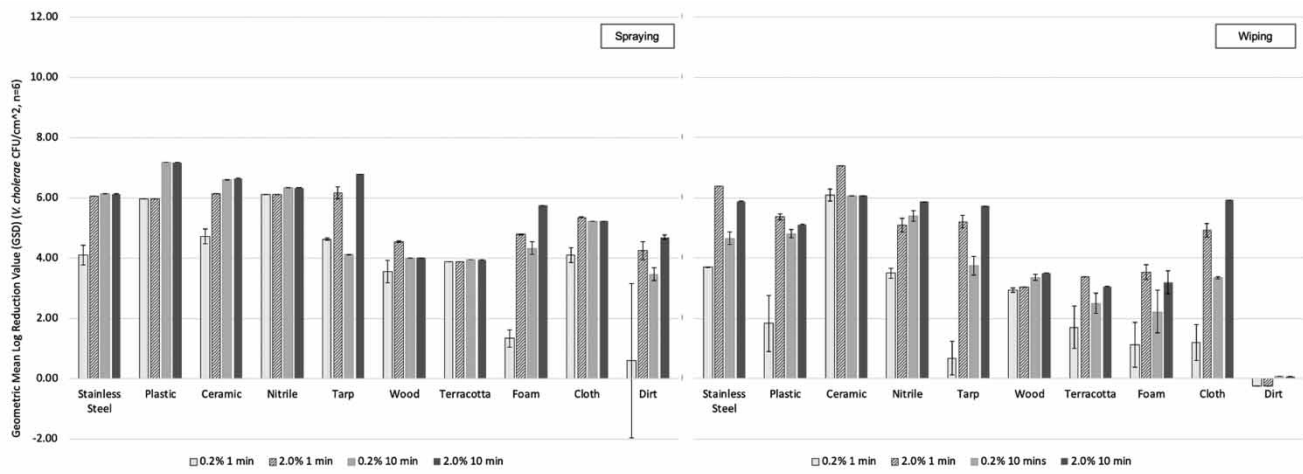

Figure 4 | Geometric mean log reduction (geometric standard deviation) of *V. cholerae* with aggregated chlorine types. The reduction values [$\log_{10}$ CFU/cm^2] are presented for each surface stratified by chlorine concentration and contact time. The error bars represent the standard error of the mean of the six aggregated samples.

V. cholerae in disinfection testing (Kolus *et al.* in press), also found no difference in the effect of chlorine compounds used to create disinfectant solutions (Gallandat *et al.* 2017). Thus, our results align with previous research and support aggregation of the results. Further support for aggregating chlorine type data is that all three chlorine types disinfect using the same compound, as all hydrolyze to form biocidal hypochlorous acid, HOCl (Iqbal *et al.* 2016; Paul Guyer 2018). A critique against aggregating chlorine data is that chlorine can be overcome by organic demand on surfaces. However, as hypothesized by others, use of high concentration solutions overcomes the chlorine demand, except for on the dirt surface carriers (Gallandat *et al.* 2017).

The exact method of chlorine application, either by spraying or wiping, impacts surface disinfection efficacy. For example, previous research has indicated that droplet size, contact time of disinfectant on the surface (related to droplet size and evaporation rate), distance from sprayer to surface, spray velocity and angle, and droplet-surface impact behavior all impact disinfectant spray efficacy (Nasr *et al.* 2016). Furthermore, variabilities in wiping healthcare surfaces, including individual differences in wiping pressure and technique; differing protocols on how to wipe and how often to wipe; differences in whether the disinfectant is applied pre-wiping, soaked into a towelette and wiped, or embedded in a pre-moistened towelette; and reuse of towelettes all impact efficacy (Williams *et al.* 2009; Carling & Bartley 2010). Even the number of times a surface was wiped was found critical in reducing bacteria on plastic (Berendt *et al.* 2011). We hypothesize that spraying reduced *V. cholerae* more than wiping in our study because a higher volume of disinfectant was in contact with the surface for a longer period of time after spraying as compared to during wiping.

We found a significantly lower reduction of *V. cholerae* on porous surfaces compared to non-porous surfaces, but higher variability in LRVs between different porous surface types and among replicates within a porous surface. This variability could be related to a lower recovery of the bacteria from porous surfaces in comparison to recovery rates from non-porous surfaces. Low recovery of *V. cholerae* on porous surfaces, such as wood and concrete, have been noted in previous surface disinfection studies (Calfee & Wendling 2013; Kolus *et al.* in press). Additionally, some evidence suggests that disinfection efficacy is actually overestimated when recovery is significantly less than 100% (Grand *et al.* 2011). Furthermore, eight studies of chlorine disinfection summarized in a recent systematic review noted that porous surfaces were more challenging to disinfect than non-porous surfaces; it was further identified that scratches on surfaces, surface roughness, and surface hydrophobicity all impacted disinfection efficacy (including one study explicitly noting that surface hydrophobicity impacted organism recovery) (Gallandat *et al.* 2020b).

Limitations to our study include: (1) the low number of replicates, although we aggregated data by removing the

effects of individual chlorine types, it is recommended to conduct testing with a minimum of triplicates in future work; (2) the wiping was conducted by one researcher, and repeatability in wiping technique was used to the extent possible, but small differences in wiping force may have occurred; (3) this work only confirmed the reduction in culturable *V. cholerae* and did not investigate the possible effects on viable but non-culturable (VBNC) cells; (4) recovery rates on some porous surfaces were low; however, these are in line with other research (Calfee & Wendling 2013; Gallandat *et al.* 2020b; Kolus *et al.* in press); and (5) no additional organic load was applied to the surfaces prior to inoculation. We do not feel these limitations impacted our results, which provide preliminary data on the efficacy of chlorine disinfection and allow for future studies to follow on with nuanced specific investigations.

Recommendations

Based on our results, we recommend the following disinfection protocols in low-resource healthcare facilities and households: (1) spray with 0.2% chlorine and allow a 10 min contact time on non-porous surfaces and porous surfaces not contaminated with cholera patient vomit or feces; (2) spray with 2.0% chlorine and allow a 10 min contact time on porous surfaces contaminated with vomit or feces; (3) wipe with 2.0% chlorine on surfaces contaminated with vomit or feces, except dirt and foam where chlorine should be applied directly to the surface; and (4) ensure that sufficient disinfectant is applied to the surface (via either method) such that the surface is visibly wet. Note, it is not recommended to spray chlorine (a) in a household with occupants inside or (b) a healthcare facility where patients or staff are present. Furthermore, we do not have evidence herein to support a recommendation on whether to implement spraying or disinfection kits at the household level.

Our recommendation for spraying with 0.2% chlorine on non-porous surfaces and 2.0% chlorine on porous surfaces aligns with a recent field evaluation of household spraying which recommended using 0.2% on household surfaces and 2.0% on heavily soiled surfaces, latrines, and kitchen/food preparation areas (Gallandat *et al.* 2020a). Additionally, our recommendation to wipe soiled surfaces with a 2.0% chlorine solution is higher than the recommended concentration for hand hygiene (Centers for Disease Control & Prevention 2018). It is, therefore, important to ensure a user has gloves to protect their hands when wiping surfaces with the higher concentration.

We recommend further efficacy research on a broader range of wiping protocols (Sattar & Maillard 2013), including spraying or pouring the disinfectant on the surface followed by wiping with a dried or prewetted towelette; the influence of different wiping materials, such as a sponge or brush; and the influence of different wiping pressures. Additionally, we recommend conducting efficacy testing on an expanded range of chlorine solution concentrations and alternate disinfectants, and examining if efficacy changes in the presence of organic load representative of bodily fluids.

This study has filled the gap on efficacy of spraying and wiping chlorine against *V. cholerae* on a range of surfaces common to low-resource households and healthcare facilities. We now know that spraying can reduce the amount of bacteria on the surface and that wiping, while not as efficacious as spraying, can also reduce the bacteria on the surface provided sufficient disinfectant is applied. However, a surface disinfection program may not necessarily be effective in an applied context, even if it is efficacious in a laboratory setting. For instance, an evaluation of three household spraying programs found that it had the potential to inactivate *V. cholerae* on household surfaces in low-resource contexts, but that effectiveness varied (Gallandat *et al.* 2020a). One program significantly reduced bacteria 30 min and 24 h after spraying; however, the second program only reduced bacteria 30 min after spraying and the third program had no reduction in bacteria. This was attributed to not spraying sufficient chlorine to fully wet the surfaces. When considering implementation, factors such as program logistics and resources, training, disinfection protocols, recontamination of surfaces, and stigma should also be examined.

CONCLUSIONS

Overall, our results fill an important gap in understanding the efficacy of various chlorine disinfection methods currently recommended or in practice. Our results support

the continued use of a 0.2% concentration chlorine sprayed on all surfaces, or wiped on most surfaces that are not heavily soiled and a 2.0% concentration applied to contaminated porous surfaces, while emphasizing the importance that surfaces are visibly wetted with chlorine to achieve disinfection. It is hoped that these results will be valuable to response organizations and governments currently implementing disinfection protocols in low-resource healthcare facilities and households, and that this efficacy data will inform current guidelines on the standard practice of disinfection in cholera outbreaks globally.

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DATA AVAILABILITY STATEMENT

All relevant data are available from an online repository or repositories (https://osf.io/d3wmg/?view_only=a60f83e7f0a94581bc96187775ea278a).

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