Journal of Water & Health

Journal of Water and Health Vol 20 No 7, 1071 doi: 10.2166/wh.2022.043

Laboratory evaluation of the efficacy of bucket chlorination guidelines at inactivating *Vibrio cholerae* for waters of varying quality

Gabrielle M. String [a,*], Annie Huang[b] and Daniele Lantagne[a]

[a] Tufts University School of Engineering, Medford, MA, USA
[b] Tufts University School of Arts and Sciences, Medford, MA, USA
*Corresponding author. E-mail: gabrielle.string@tufts.edu

GMS, 0000-0002-0266-923X

ABSTRACT

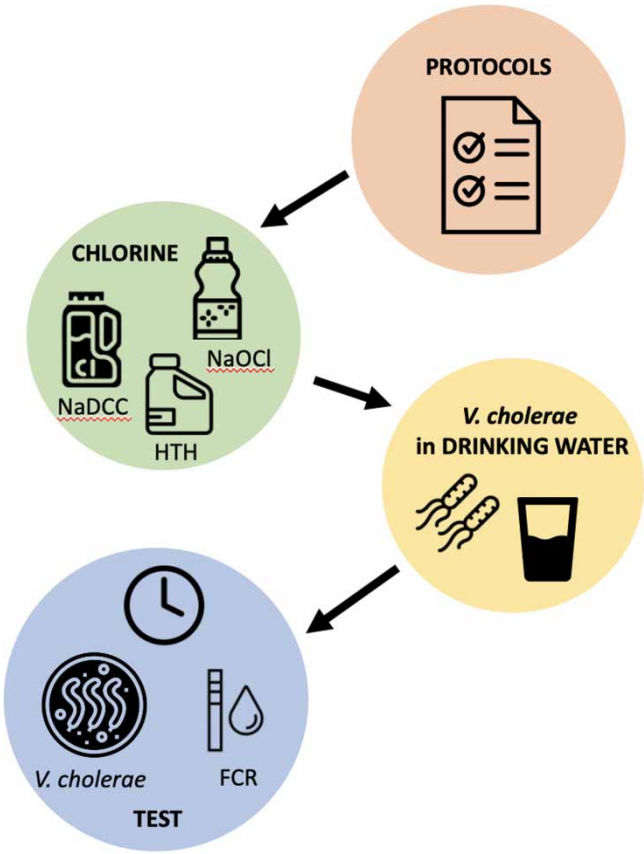
Bucket chlorination, where chlorine is dosed directly into water collection containers, is a point-of-source water treatment intervention commonly implemented in cholera outbreaks. There is little previous data on chlorine efficacy against *Vibrio cholerae* in different waters and appropriate dosage regimes. We evaluated *V. cholerae* reduction and free chlorine residual (FCR) in waters with four turbidities (1/5/10/50 NTU), two total organic carbon (TOC) concentrations (0.4, 1 mg/L), and two dosing schemes (fixed-dose of 2 or 4 mg/L, variable-dose based on jar testing) treated with three chlorine types (HTH, NaOCl, NaDCC). We found that chlorine was efficacious at reducing *V. cholerae* by ≥2.75 to ≥3.63 log reduction value (LRV); variably dosed reactors were dosed higher, met ≥0.5 mg/L FCR at 30 min, and had higher LRVs (p=0.024) than fixed doses; and low TOC reactors had more samples ≥0.2 mg/L FRC at 4 h (p=0.007). Our results are conservative, as internationally recommended additives to create test water increased chlorine demand, highlighting the challenge of replicating field conditions in laboratory testing. Overall, we found that chlorine can efficaciously reduce *V. cholerae*; we recommend further research on appropriate chlorine demand for test waters; and we recommend establishing appropriate chlorine doses based on source water and taste/odor acceptability in bucket chlorination programs.

Key words: chlorine, cholera, dosage, emergency, water quality, water treatment

HIGHLIGHTS

- Bucket chlorination is a point-of-collection emergency water treatment program.
- We tested the efficacy of chlorine dosing guidelines against *Vibrio cholerae* in waters of varying turbidity and total organic carbon.
- Chlorine efficaciously reduces *V. cholerae* and is recommended at appropriate doses in bucket chlorination programs.
- Chlorine decayed rapidly – test water chlorine demand may be higher than natural waters.

GRAPHICAL ABSTRACT



INTRODUCTION

Cholera is caused by the ingestion of the *Vibrio cholerae* bacterium and can result in acute diarrhea and vomiting, leading to severe dehydration and shock (Sack *et al.* 2004). In 2019, 2,485 estimated deaths and 128,121 cases were reported to the World Health Organization (WHO) globally as a result of cholera infection (WHO 2019a, 2019b). Furthermore, it was estimated that 1.3 billion people are at risk for cholera in endemic countries (Ali *et al.* 2015). To reduce cholera deaths by 90% and eliminate cholera in 20 countries by 2030, the Global Task Force on Cholera Control launched a strategy that includes early detection and containment of outbreaks (Global Task Force on Cholera Control 2017; WHO 2019b).

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). To contain outbreaks and reduce the risk of cholera and other waterborne diseases, chlorine-based disinfection of drinking water is widely implemented (Branz *et al.* 2017). Advantages of treating drinking water with chlorine in chlorine response include its efficacy (*V. cholerae* has a CT factor of 0.5 mg min/L (Centers for Disease Control and Prevention n.d.; Morris *et al.* 1996)), ease of use, cost-effectiveness, availability, ease of verification, and maintenance of chlorine residual to protect stored water from recontamination (Lantagne & Clasen 2012). Challenges to implementing chlorine disinfection include differing guidelines on dosage and free chlorine residual (FCR) targets, technical knowledge needed to dose and monitor FCR, taste and odor acceptability of chlorine, and ensuring chlorine residual until time of consumption (Branz *et al.* 2017; Murphy *et al.* 2018; Spina *et al.* 2018; Sikder *et al.* 2020).

Bucket chlorination is a widely used point-of-collection chlorine-based water treatment intervention (Dunoyer n.d.; UNICEF n.d.; Lamond & Kinyanjui 2012; Olson *et al.* 2017). In bucket chlorination interventions, a trained agent is stationed at water collection points to dose chlorine directly into users' water containers during collection. Despite

implementations dating back to the 1930s (Robertson & Pollitzer 1939) and the current prevalence of bucket chlorination in outbreak response (especially in case-area targeted interventions (Finger *et al.* 2018; Ratnayake *et al.* 2021)), bucket chlorination was identified in an evidence review as a commonly implemented but severely under-researched intervention (Yates *et al.* 2018). In particular, little is known about the laboratory efficacy of chlorination against *V. cholerae* in waters with varying turbidity, TOC, and chlorine demand with varying doses of different chlorine types. Water quality parameters such as temperature, TOC, and total dissolved solids are criteria that are adjusted when preparing test water in laboratory studies of chlorine efficacy (WHO 2014). However, bucket chlorination programs primarily utilize easy-to-measure, in-field water quality parameters, including source water turbidity and pH, to determine dosing protocols (WHO 2017).

More specifically, an obstacle in implementing bucket chlorination programs is reconciling conflicting recommendations. Dose recommendations are either empirically determined for each water source via jar testing (also known as the Modified Horrocks' Method) or fixed based on source water quality.

For empirically determined dose schemes, frequent jar testing is recommended at the source to select the dose that provides FCR 30 min after dosing of 0.2–0.5 mg/L if pH<8 (Van Den Noortgate & Maes 2010), 0.3–0.5 mg/L (Lamond & Kinyanjui 2012), 0.5–1.0 mg/L (ACF 2013), or 0.5–1.0 mg/L if water <5 NTU (UNICEF n.d.), or 0.5 mg/L if pH≤8 and 1.0 mg/L if pH>8 (Olson *et al.* 2017). A scoping review of WASH guidelines for cholera prevention and control found that eight international guidelines recommend >0.5 mg/L FCR 30 min after treatment for source water treatment programs (D'Mello-Guyett *et al.* 2020).

Fixed-dose schemes are more commonly recommended for household water treatment, and sometimes also used for bucket chlorination. For example, a fixed dose of 2.5 mg/L of chlorine is recommended for bucket chlorination (Johns Hopkins University and International Federation of Red Cross and Red Crescent Societies 2008), and WHO recommends a fixed dose of 2 mg/L for clear water with turbidity <10 NTU and a double dosage of 4 mg/L for turbid water >10 NTU for household water treatment (Lantagne 2008; WHO 2017). Additionally, results from lab and field testing of chlorine dosages recommended a chlorine dose of 1.88 mg/L in improved/low turbidity sources and 3.75 mg/L in unimproved/high turbidity sources (Wilhelm *et al.* 2017).

Studies have reported on bucket chlorination effectiveness in the field, using FCR in households as the outcome measure. In a bucket chlorination program in Cox's Bazar refugee camps, 71% of households surveyed had ≥0.2 mg/L FCR in stored water, with an empirically determined dose established once at the program start (Sikder *et al.* 2020). In Chad, bucket chlorination implemented in response to a Hepatitis E outbreak resulted in 43% of surveyed households with ≥0.2 mg/L FCR in stored drinking water (Spina *et al.* 2018), with higher percentages of FCR found when containers were empty before water collection, and if chlorinated water was collected within 6 h of the survey. In Cameroon, bucket chlorination was implemented during a cholera outbreak by adding a 1% chlorine solution prepared from calcium hypochlorite into water collection containers at an empirically determined dose based on source water quality (Murphy *et al.* 2018). While coverage was high, with 80% of surveyed households receiving bucket chlorination in the previous 24 h, only 8% had stored water with FCR ≥0.2 mg/L.

Overall, evidence is needed to understand if bucket chlorination can be efficacious to remove *V. cholerae*, as mediated by chlorine type and concentration, source water turbidity and total organic carbon (TOC) content, and variable and fixed-dose protocols. The goal of this study was to fill these evidence gaps.

MATERIALS AND METHODS

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

Test matrix

We selected four turbidities (1, 5, 10, and 50 NTU) and two TOC levels (0.4 and 1 mg/L) to represent a range of natural water qualities following recommendations for preparation of test waters (WHO 2014; 'Personal communication between authors and members of the WHO Household Water Treatment Testing Scheme' 2017) and observed chlorine demand in pre-testing (Figure 1). We included all three chlorine compounds commonly available in humanitarian contexts (Wells *et al.* 2016), including sodium hypochlorite (NaOCl), high-test calcium hypochlorite (HTH), and sodium dichloroisocyanurate

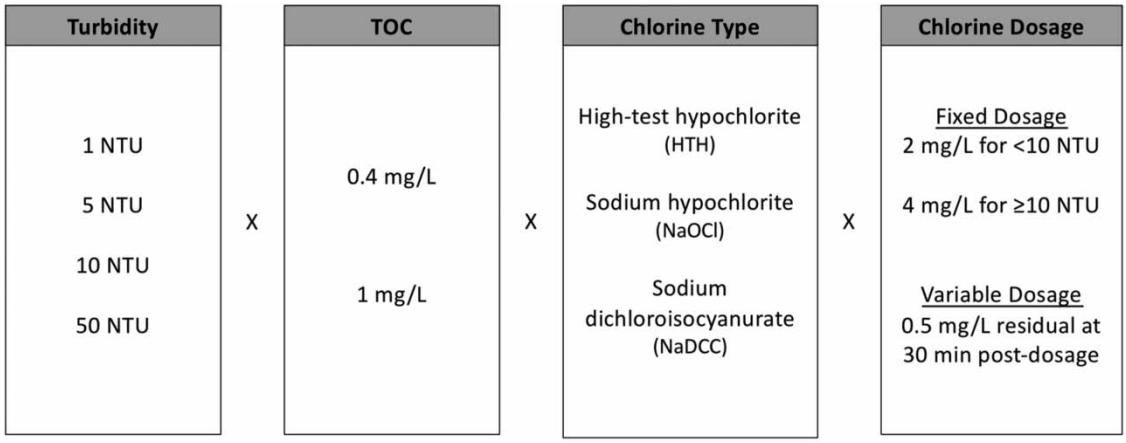


Figure 1 | Test matrix.

(NaDCC). Water was treated with either a variable dose that provided FCR>0.5 mg/L 30 min after treatment, or a household water treatment fixed dose dependent on turbidity (2 mg/L for <10 NTU and 4 mg/L for ≥10 NTU).

Preparation of test waters

Test water was prepared in 20 L sterile containers by buffering 8 L 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', with 2 L of phosphate-buffered saline (PBS) (pH=7.6). Tannic acid (ACROS Organics, Fair Lawn, NJ, USA) was added to the buffered Milli-Q to achieve the appropriate final test concentration for TOC addition (0.4 or 1.0 mg/L).

For turbidity addition, sediments were collected by removing 5 cm of material from the Mystic River creek-bed (Medford, MA, USA), and collecting the 10 cm layer beneath. In the laboratory, these creek-bed sediments were sieved through a 18×14 mesh, rinsed, dried at 100 °C for 72 h in an oven, and sterilized by autoclaving. Sediment solid organic carbon content was measured by processing total and inorganic carbon content samples (where Total–Inorganic=Organic Carbon) using a Shimadzu TOC-L+SSM-5000A Analyzer (Shimadzu Corporation, Kyoto, Japan). Total and inorganic carbon were calculated against standard curves of ranges 0–30 and 0–3 mg carbon, respectively. A predetermined mass of sediments was mixed in a sterile Erlenmeyer flask with 100 mL of Milli-Q; on average, this was 0.598 g for 1 NTU water, 7.563 g for 5 NTU water, 10.81 g for 10 NTU water, and 21.77 g for 50 NTU water. This emulsion was then added to the 10 L of test water, and placed on a stir plate for 30 min to ensure prepared test water consistency.

Test water pH, TDS, and temperature were tested using an Oakton PC700 benchtop meter (Oakton, Vernon Hills, IL, USA). Test water turbidity was confirmed to be within 10% of the target with a calibrated LaMotte 2020we turbidimeter (LaMotte, Chestertown, MD, USA), and adjusted and retested if necessary. One opaque sterile bottle was filled with 1 L of test water, labeled as a negative control and set aside until testing. On variable chlorine dose test days, 100 mL of test water was dispensed into each of 12 sterile glass bottles (125 mL) and set aside for jar testing described below.

V. cholerae (El Tor N16961, ATCC 39315) was streaked from stock 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. To prepare an exponential phase culture each test day, the first 10 mL of Tryptic Soy Broth (TSB) in a sterile Falcon tube was inoculated by isolating a colony from the streak plate. The Falcon tube was incubated at 35 °C on a shake plate for 12–18 h. This overnight culture was then diluted (1:25) in 50 mL of sterile TSB and incubated at 35 °C on a shake plate until a concentration of ~10^{10} cells/mL was reached, as estimated using a spectrophotometer (OD=600 nm) (GeneQuant 100, Biochrom Division of Harvard Bioscience, MA, USA).

To remove chlorine demand caused by the TSB, the culture was washed in PBS. The Falcon tube was first placed in an Eppendorf 5810R centrifuge (Eppendorf, Hamburg, Germany) at 4 °C and spun at 4,000 rpm for 5 min. In a biosafety cabinet, the TSB was drawn off using a serological pipette taking care not to disturb the pellet. Then, 20 mL of PBS was added to the Falcon tube, and the tube was continuously vortexed at medium speed for 5 min to resuspend the solution. The culture was centrifuged once more, supernatant drawn off, and the pellet resuspended in 20 mL of fresh PBS. The OD600 of the

suspension was checked to confirm no significant loss of cells from washing. Then, the suspension was used to inoculate the prepared test water at a concentration of 10^6 cells/100 mL; the test water was stirred for 15 min. Test water (1 L) was dispensed into seven opaque sterile bottles, hereafter termed reactors, and used immediately in testing.

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 using iodometric titration (Method 8209, Hach Company, CO, USA) to be within 10% of the target concentration, 1.0% chlorine (10,000 mg Cl/L). Solutions were adjusted and retested if necessary. The chlorine concentration of each solution was recorded on each test day and used to calculate the volume of chlorine dosed into the water.

For variable chlorine doses, a jar test was conducted before water treatment following Médecins Sans Frontières methods (Van Den Noortgate & Maes 2010). Then, 4 of 12 reactors containing prepared test water were labeled for each chlorine type (NaOCl, HTH, and NaDCC). Each chlorine type was dosed into each of the four reactors in increasing amounts, where the range of doses tested varied according to water turbidity and TOC. For example, in 1 NTU, low TOC water the doses were 0.5, 1, 2, and 4 mg/L, while in 50 NTU, high TOC water the doses were 6, 7, 8, and 9 mg/L. FCR was tested 30 min after chlorine addition using a calibrated colorimeter and DPD-1 instrument grade tablets (LaMotte 1200, Chestertown, MD, USA). The dose selected for testing corresponded to the reactor with FCR closest to 0.5 mg/L at 30 min (without going below).

Water treatment tests and sample processing

Of the eight test water reactors, one without *V. cholerae* was labeled a negative control, and one was a positive control. The remaining six reactors (one for each chlorine type in duplicate) were labeled by chlorine treatment: NaOCl, HTH, or NaDCC. For variable-dose tests, the volume of chlorine determined from jar testing was pipetted into each corresponding reactor. For fixed-dose tests, a pre-calculated volume of chlorine was pipetted into the corresponding reactor at doses of 2 mg/L for $<$10 NTU and 4 mg/L for $\geq$10 NTU. At 30 min, and 4, 8, and 24 h after dosing, samples were collected from each treated reactor and tested for FCR, as described previously. At 30 min and 24 h after dosing, 200 mL of test water was poured from each of the eight reactors into pre-labeled Whirl-Pak® Thio-Bags® (Nasco, Fort Atkinson, WI, USA), containing a sodium thiosulfate tablet to neutralize chlorine residual, and stored on ice for no more than 2 h until processing.

Samples were filtered by passing 100 mL of water from each bag across a 0.22 μm polycarbonate membrane filter (MilliporeSigma, Burlington, MA, USA). Membranes were each placed into a labeled 50 mL Falcon tube containing 12 mL of PBS. Tubes were vortexed for 5 min to detach *V. cholerae* from the membrane surface and resuspended in PBS following pre-existing protocols (Huq *et al.* 2012). Appropriate dilutions of each sample were prepared in 900 μL of PBS in sterile Eppendorf tubes and kept on ice until plating. Samples were spread (250 μL) onto thiosulfate-citrate bile salts agar (BD Difco, East Rutherford, NJ, USA), dried for 15 min, then inverted and incubated at 35 °C for 24 h when *V. cholerae* colonies were enumerated and recorded.

Data analysis

Data were entered and analyzed in Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA). FCR readings below the manufacturer advertised minimum detection limit of 0.05 mg/L were recoded to 0.00 mg/L. Replicate FCR values were averaged and plotted on decay curves for each sample combination. CT factors were calculated at each time point by multiplying the average disinfectant concentration (FCR) between exposure time points by exposure time, in minutes, and summed. They were then compared with existing CT factors for *V. cholerae* (Centers for Disease Control and Prevention n.d.; Morris *et al.* 1996). Pearson's Chi-squared tests were used to determine if chlorine type, dose scheme, or TOC level were associated with cut-off values of $\geq$0.5 mg/L FCR at 30 min and $\geq$0.2 mg/L FCR at 4 h (WHO 2017; D'Mello-Guyett *et al.* 2020).

Geometric mean *V. cholerae* concentrations were calculated for each cultured sample, accounting for dilutions, and reported in CFU/100 mL. Plates with zero values were replaced with half the theoretical detection limit, 0.5, which corresponded to 24 CFU/100 mL in the least diluted samples; plate counts above the detection limit were assigned a value of 250 CFU. Replicates were averaged, and log reduction values and standard errors were calculated. A Welch's *t*-test was

performed to compare mean LRVs between the fixed- and variable-dose groups, between chlorine types within the fixed and variable-dose groups, between high and low TOC water, and between high and low TOC water by dose level. By chlorine dose scheme, Kruskal–Wallis tests were used to determine if LRVs were different by turbidity; where there were significant differences a *post-hoc* Dunn's test with a Bonferroni correction was used to determine which turbidity levels varied.

RESULTS

In total, 48 tests were run in duplicate with positive and negative controls, resulting in 128 test bottles sampled. Test waters were spiked with 1.60×10^{8}–1.32×10^{9} CFU/mL *V. cholerae* and positive controls ranged from 3.23×10^{5} to 9.06×10^{6} CFU/100 mL 30 min after dosing to 3.10×10^{7}–1.45×10^{9} CFU/100 mL 24 h after dosing.

Chlorine dose results

Reactors with a fixed chlorine dose were dosed as described in Methods. Reactors with a variable dose were dosed based on the outcome of jar tests (Table 1). For all chlorine types and turbidities, doses for reactors with high TOC were higher than low TOC reactors.

Table 1 | Jar test results for variably dosed reactors

			Free chlorine residual (mg/L)		
NTU	**TOC**	**Dose (mg/L)**	**HTH**	**NaOCl**	**NaDCC**
1	Low	0.5	0.12	0.19	0.17
		1.0	**1.05**	0.41	0.44
		2.0	1.3	**1.47**	**1.22**
		4.0	2.37	3.08	2.63
	High	2.0	0†	0†	0†
		4.0	0.38	0.15	0.07
		5.0	**0.56**	**0.82**	0.33
		6.0	1.57	1.49	**2.34**
5	Low	0.5	0†	0.08	0†
		1.0	0.28	0.43	0.25
		2.0	**0.97**	**1.15**	**1.15**
		4.0	2.41	2.98	2.81
	High	2.0	0†	0†	0†
		4.0	0.29	**0.55**	0†
		5.0	**1.13**	1.38	**1.11**
		6.0	1.69	1.88	1.89
10	Low	1.0	0.24	0.23	0.5
		2.0	**0.99**	**0.92**	**1.71**
		4.0	2.43	2.2	2.51
		5.0	2.83	2.95	>4.0††
	High	2.0	0†	0†	0†
		4.0	0.27	**0.56**	**2.03**
		5.0	**0.99**	1.29	3.04
		6.0	1.79	1.93	>4.0††
50	Low	4.0	0.06	0†	0.09
		5.0	0.03	0.07	**0.82**
		6.0	0.16	0.23	1.53
		7.0	**0.68**	**0.89**	2.71
	High	6.0	0†	0†	0.3
		7.0	0†	0†	**1.25**
		8.0	0.23	0.32	2.55
		9.0	**0.58**	**0.67**	>4.0††

Bolded FCR results indicate the reactors with FCR closest to 0.5 mg/L without going below. The corresponding dose was selected in testing.

†FCR at this time point was below the detection limit of the instrument and recoded to 0 mg/L.

††FCR was above the detection limit of the instrument and has been recoded to >4.0 mg/L.

Free chlorine residual results

FCR decayed in all test samples over time (Figure 2). In fact, only two fixed-dose samples and six variable-dose samples had detectable FCR at 24 h after dosing, which differs from previous literature and is attributed to the high organic load of *V. cholerae* in the test reactors.

For fixed-dose samples, by chlorine type, no significant differences were observed between samples that met ≥0.5 mg/L at 30 min (p=0.497) and between samples that met ≥0.2 mg/L at 4 h (p=0.324). Furthermore, for variably dosed samples, by chlorine type, no significant differences were observed between samples that met ≥0.2 mg/L at 4 h (p=0.437). Additionally, for fixed-dose samples at 30 min, no chi-square test could be completed as all NaOCl and NaDCC samples were ≥0.5 mg/L. Overall, differences in FCR were not driven by chemical composition differences between the three chlorine types tested, HTH, NaOCl, and NaDCC.

No significant differences were observed by TOC level for samples that were able to sustain ≥0.5 mg/L FCR at 30 min (p=0.077); however, low TOC water had significantly more samples ≥0.2 mg/L at 4 h than high TOC water (p=0.007). Additionally, significantly more samples treated with a variable dose met ≥0.5 mg/L at 30 min (p<0.001) and ≥0.2 mg/L at 4 h (p=0.007) than samples treated with a fixed dose.

For all reactors, CT factors were above 0.50 mg min/L, the minimum for *V. cholerae* inactivation 30 min after dosing (Table 2). Furthermore, at 30 min, 33/48 reactors had CT factors ≥40 mg min/L, the minimum for rugose strain *V. cholerae* inactivation, representing likely residual protection even against chlorine-resistant strains. No high TOC reactor with a fixed dose achieved a CT factor of ≥40 mg min/L; all high TOC reactors with a variable dose did meet this threshold.

V. cholerae results

At 30 min and 24 h, *V. cholerae* was reduced by ≥2.75 LRV for all reactors treated with a fixed chlorine dose and ≥3.63 LRV for all reactors treated with a variable dose (Figure 3); this difference was significant (p=0.024). Of fixed-dosed reactors, 3 with low TOC and 10 with high TOC had detectable *V. cholerae* 30 min after treatment; of variably dosed reactors 1 each with low and high TOC had detectable *V. cholerae*.

When comparing chlorine type for both fixed and variable doses, no differences were observed in *V. cholerae* LRV between HTH and NaOCl (p=0.913 fixed, p=0.707 variable), HTH and NaDCC (p=0.805 fixed, p=0.961 variable), or NaOCl and NaDCC (p=0.722 fixed, p=0.671 variable). Therefore, chlorine type did not impact the efficacy of disinfection against *V. cholerae*.

When comparing high TOC to low TOC waters, differences in *V. cholerae* LRV were not seen (p=0.229), even when taking the dose type (fixed/variable) into consideration. There were significant differences in *V. cholerae* LRV within fixed-dose samples (p=0.004) and within variable-dose samples (p=0.032) when grouped by turbidity. *Post-hoc* analysis showed that for fixed-dose samples, this was driven by differences in *V. cholerae* LRV for waters of 1 NTU and 5 NTU (p=0.046) and 5 NTU and 50 NTU (p=0.005); similarly, for variable-dose samples, this was true when comparing 5 NTU and 50 NTU (p=0.022). Ultimately, as most of the samples had no *V. cholerae* detected at 30 min and 24 h, the differences in LRV are likely driven by the differences in the starting concentration of *V. cholerae* in test water each day prior to treatment and not driven by water quality characteristics. Furthermore, the increased LRVs from 30 min to 24 h are due to the growth of *V. cholerae* in the positive control reactors over the course of the experiment.

When analyzing carbon content of the sediments, no sample registered a measurable concentration of inorganic carbon. Therefore, the total carbon content of the sediments was assumed to be entirely organic carbon. For the nine samples, carbon content in the sediment ranged from 2.19 to 2.55%. Multiplying the average sediment carbon content by the average mass of sediment added to the prepared test water for each turbidity level results in an additional 1.4, 17.9, 25.5, and 51.4 mg/L in 1, 5, 10, and 50 NTU waters, respectively.

DISCUSSION

We tested the efficacy of chlorine treatment against *V. cholerae* in waters of varying quality using both variable and fixed dosing protocols. In our testing, we evaluated three different chlorine types, four turbidities, two TOC concentrations, and two types of dose schemes. Overall, we found that chlorine can efficaciously reduce *V. cholerae*, even under our test conditions which were more challenging than natural waters, and that the rapid decay of FCR seen in our study over 24 h highlights the challenge of designing laboratory efficacy tests that reflect real-world conditions. Specifically, we found that (1) *V. cholerae* was reduced after chlorine treatment with an appropriate dose of all chlorine types, with significantly more

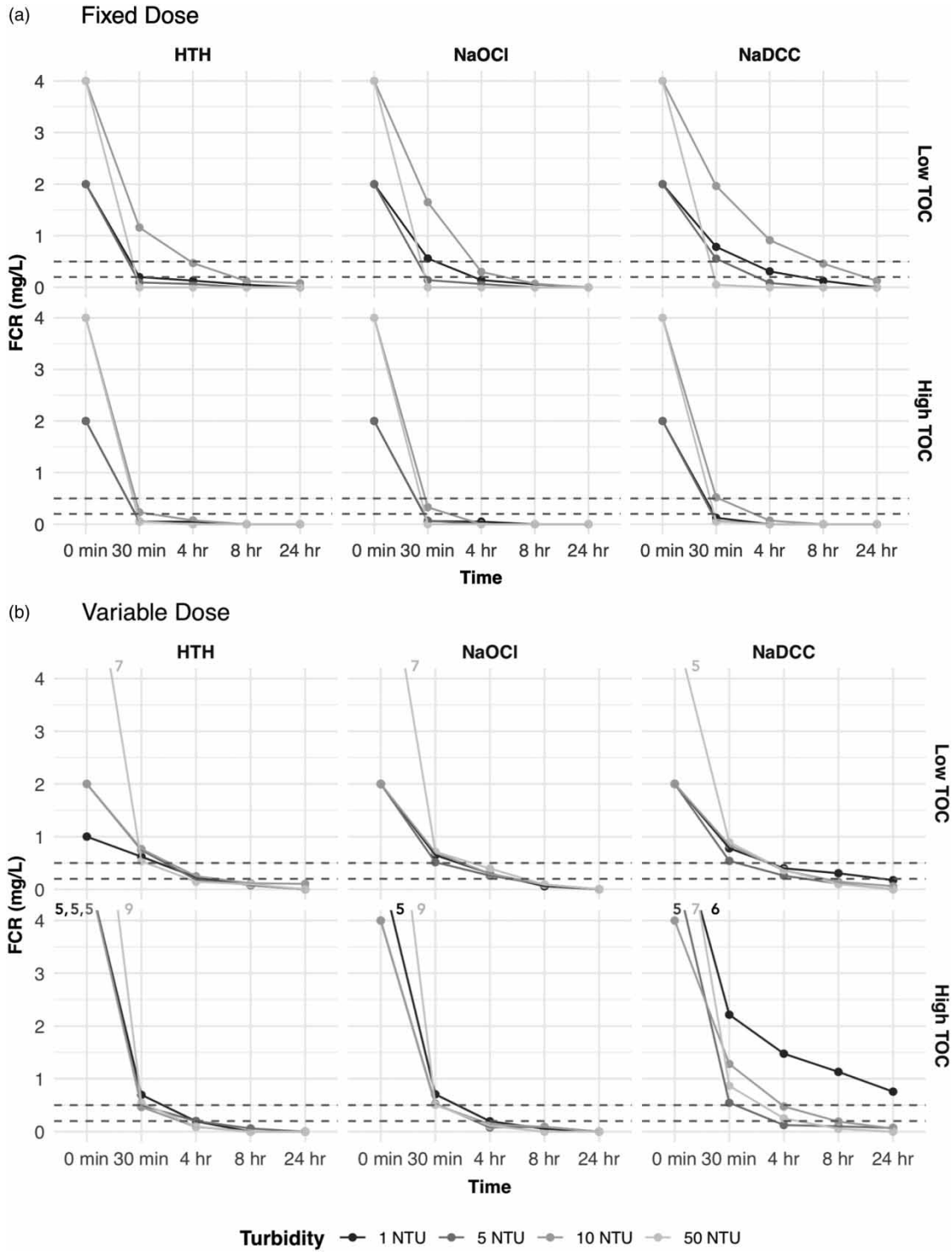


Figure 2 | FCR decay curves for (a) fixed chlorine dose samples and (b) variable chlorine dose samples. Dashed lines mark the 0.5 and 0.2 mg/L FCR standards. Note: initial doses are plotted at the 0 min timepoint. Variable doses are indicated on the plot next to the corresponding FCR data where the starting dose exceeds 4 mg/L.

samples ≥0.5 mg/L FCR 30 min after a variable dose than a fixed dose and significantly higher LRVs from variable doses than fixed doses; (2) reactors with a lower added TOC had significantly more samples ≥0.2 mg/L at 4 h although no differences were observed in LRV; (3) turbidity could have been a driver of observed differences in *V. cholerae* reduction, and the

Table 2 | CT factors in mg min/L for each unique reactor (n=2, averaged duplicates)

		HTH				NaOCl				NaDCC			
TOC	NTU	30 min	1 h	4 h	24 h	30 min	1 h	4 h	24 h	30 min	1 h	4 h	24 h
Fixed-dose CT factors (mg • min/L)													
Low	1	33.0	67.6	89.2	113	38.4	111	135	164	41.7	156	208	268
	5	31.4	48.2	56.0	56.0†	32.1	53.6	61.4	61.4	38.3	105	115	115†
	10	77.4	248.5	319	415	84.7	289	333	367	89.4	391	556	837
	50	60.0	60.0†	60.0†	60.0†	60.0	60.0†	60.0†	60.0†	60.7	66.0	66.0†	66.0†
High	1	30.7	41.2	47.2	47.2†	30.9	42.4	48.4	48.4†	31.8	45.0	45.0†	45.0†
	5	30.8	36.6	36.6†	36.6†	31.0	38.4	38.4†	38.4†	30.9	37.2	37.2†	37.2†
	10	63.4	95.4	104	104†	64.9	99.6	99.6†	99.6†	67.8	129	138	138†
	50	60.7	66.0	66.0†	66.0†	60.0	60.0†	60.0†	60.0†	60.7	66.0	66.0†	66.0†
Variable-dose CT factors (mg • min/L)													
Low	1	24.3	56.8	94.0	132	39.7	48.6	91.8	120	41.7	108	193	423
	5	41.1	78.9	111	152	37.7	47.1	89.1	132	38.1	113	157	210
	10	41.4	115	159	264	40.5	52.0	98.2	141	42.6	142	203	301
	50	113	133	162	207	115	122	180	223	88.4	145	201	249
High	1	85.4	178	202	202†	85.5	180	210	236	123	510	823	1,728
	5	82.4	155	186	215	67.8	131	151	195	83.1	153	180	262
	10	81.9	140	151	151†	67.6	135	163	206	79.2	263	343	463
	50	143	210	221	221†	142	207	220	220†	117	235	271	298

†FCR at this time point was non-detect.

addition of sediments to mimic turbidity increased the total carbon added to the test waters. Each of these is further described below.

While *V. cholerae* concentrations were reduced with both dose schemes, it is not unexpected that a variable dose was able to provide the outbreak recommended FCR of >0.5 mg/L at 30 min more reliably, as the dose of chlorine added to the water was greater than fixed doses. Thus, the LRV for the variably dosed samples was higher than the fixed-dosed samples. However, while no reactors had an FCR that exceeded recommended maximums of 4.0–5.0 mg/L (US EPA 2015; WHO 2017), five had FCR ≥1.0 mg/L which could exceed acceptable taste and odor limits in some contexts (Branz *et al.* 2017). Furthermore, not finding any disinfection differences between the three types of chlorine tested is consistent with previous research that found similar bactericidal properties between different products containing the same amount of available chlorine (Coates 1985). Different formulations provide varying advantages and disadvantages in the manufacture, transport, and storage of chlorine compounds which impact the stability of resultant chlorine solutions. When preparing chlorine solutions for bucket chlorination, it is crucial to understand that the stability can decrease the amount of available chlorine; shelf-life of 0.5% chlorine solutions can range from 2 days (NaDCC) to >30 days (HTH and stabilized NaOCl) (Iqbal *et al.* 2016).

In general, our results are consistent with previous testing of a fixed dose of NaOCl and NaDCC in reactors with varying turbidity and added TOC (Wilhelm *et al.* 2017; Gallandat *et al.* 2019). However, our FCR decayed more rapidly by the 24-h timepoint. While it is possible our reactors decayed faster than those in Gallandat *et al.* because of chlorine demand from the *V. cholerae* in test waters, we hypothesize our use of tannic acid (as compared with the TOC stock solution used by Gallandat *et al.*) for TOC load in our reactors is the cause of the chlorine demand. Although our tannic acid TOC levels of 0.4 and 1.0 mg/L were lower than average natural water globally ('Personal communication between authors & members of the WHO Household Water Treatment Testing Scheme' 2017), this hypothesis is supported by two pieces of evidence. First, in our study, low TOC water had more samples at 4 h meet the ≥0.2 mg/L FCR standard (as compared with high TOC water). Second, as manufactured TOC standards do not exert chlorine demand on test waters (Gallandat *et al.* 2019), the WHO recommends using tannic acid for low added TOC (1.05 ± 0.95 mg/L) and humic acid for high added TOC (15 ± 5 mg/L) in their protocol for prepared laboratory waters to test household water treatment technologies (WHO 2014). In pre-testing, we identified two challenges using humic acid: humic acid added color to the test water which interfered with turbidity measurements; and *V. cholerae* was inactivated at a higher rate when spiked into water containing humic acid than tannic acid (before the addition of chlorine). These differences are attributed to the fact tannic acid is a polyphenol and

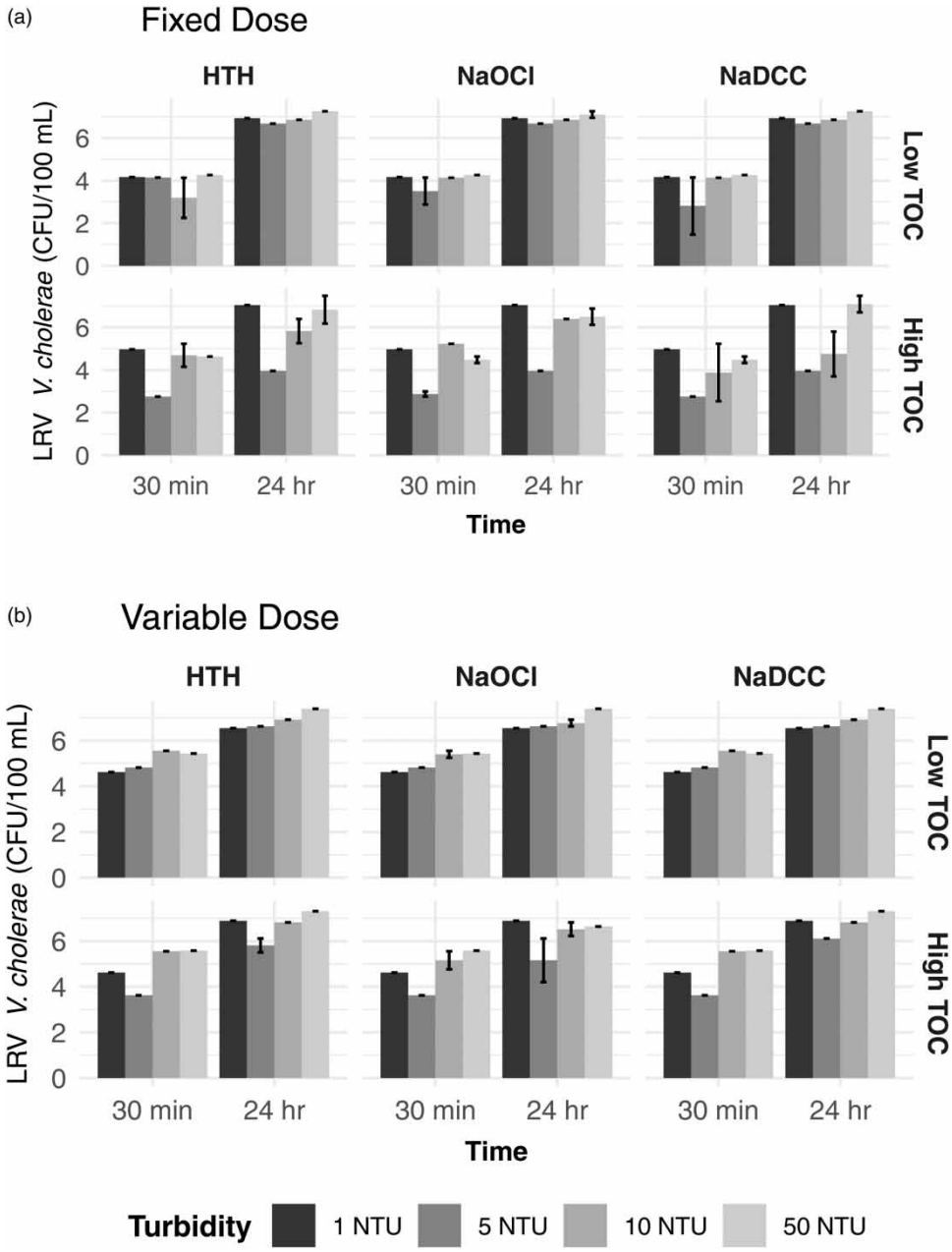


Figure 3 | *V. cholerae* LRVs stratified by chlorine type, TOC level, and turbidity for (a) fixed chlorine dose samples and (b) variable chlorine dose samples.

humic acid contains both phenolic and carboxylic substitutes. To keep the organic material the same for the low and high TOC reactors and to remove the observed challenges of using humic acid, we used tannic acid to add TOC to all our prepared test waters. However, the addition of TOC was calculated by the weight of carbon in the tannic acid using the gravimetric method recommended in the WHO Household Water Treatment Testing Scheme, not the chlorine demand of the acid.

There were small differences observed in *V. cholerae* LRV when considering the turbidity of the reactors. However, because most reactors had no *V. cholerae* detected 30 min and 24 h after dosing, it is inconclusive whether these differences are due to the turbidity level or differences in the influent water concentration. Additionally, the higher sediment mass added to reactors to make higher turbidity water also resulted in additional organic carbon load to the reactors. The additional organic carbon load added to 50 NTU reactors averaged 50 mg/L more than in the 1 NTU reactors. It is likely that this created additional

chlorine demand and corroborates previous research that found decreased FCR among reactors using creek-bed sediments but not those using kaolin clay for turbidity (Gallandat *et al.* 2019).

Our results highlight the challenges of testing chlorine efficacy in the laboratory setting. We are not the first to struggle with replicating field level chlorine dosage and FCR decay using test waters in the laboratory ('Personal communication between authors and members of the WHO Household Water Treatment Testing Scheme' 2017; 'Personal communication between authors and laboratory scientists at the US CDC's Waterborne Disease Prevention Branch' 2020). Laboratory water used for testing household water treatment devices was optimized for testing filters and other devices, as chlorination was approved for use before testing protocols existed. Thus, protocols to prepare test waters have not been optimized for testing chlorine efficacy in the laboratory. Further research quantifying how individual additives (turbidity, TOC, microorganisms) in laboratory test waters impact chlorine demand, FCR maintenance, and thus chlorine efficacy is needed across chlorine types and doses. Appropriate test waters that mimic natural chlorine demand (without adding unnaturally high chlorine demands) are needed for testing chlorine efficacy.

The main field-based recommendation from our research is: fixed *or* variable chlorine dose protocols can be used in bucket chlorination programs. In situations where a fixed dose (based on turbidity) is used due to context-specific conditions, we recommend implementers measure FCR of dosed containers at 30 min following the guidelines of chlorine product manufacturers and previous researchers to determine if the fixed dose is efficacious at maintaining FCR (Murphy *et al.* 2018; CAWST 2021). Current detailed guidelines for establishing variable dosages using jar testing are available for implementers (Olson *et al.* 2017). In all dosage testing, it is recommended responders are equipped with FCR test kits and turbidity to determine if pre-treatment is necessary before disinfection with chlorine. Additionally, responders should note the high doses possibly needed to achieve FCR of 0.5 mg/L in poor quality water (particularly with variable doses) may exceed recommended dose and taste/odor thresholds (US EPA 2015; WHO 2017).

Limitations to our study include (1) the low number of replicates; (2) the use of tannic acid to generate TOC, as described above; (3) the use of laboratory prepared water instead of natural waters that led to increased chlorine demand of waters, as described above; and (4) subsequent use of higher chlorine doses in variably dosed reactors that exceed recommended thresholds (WHO 2017). As our results are in water with high chlorine demand, the efficacy results presented herein are considered conservative.

Lastly, we recommend evaluating the effectiveness of bucket chlorination programs as implemented in outbreak response to understand how programs are realistically implemented and if a variable dose reduces *V. cholerae* and maintains FCR in household containers.

CONCLUSION

Overall, our results fill an important gap in understanding the efficacy of various recommended chlorine dose guidelines for bucket chlorination. Our results support previous work reporting that chlorine inactivates *V. cholerae* and the use of an appropriate chlorine dose determined by source water quality. Additionally, this work contributes to the discussion on how to test chlorine efficacy in laboratory prepared waters. It is expected that these results will help responders to more effectively implement emergency water treatment programs and that this data will help to clarify current guidelines on dose selection in outbreak contexts.

ACKNOWLEDGEMENTS

We would like to thank Chen Liu and Jason Hnatko for training on the SSM-5000A TOC-L/TOC-V Lab TOC analyzer and Anthonia Ogudipe for assistance processing samples on the machine.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

ACF 2013 *EAU – Assainissment – Hygiene dans la Lutte Contre le Cholera 107*.

Ali, M., Nelson, A. R., Lopez, A. L. & Sack, D. A. 2015 Updated global burden of cholera in endemic countries. *PLoS Negl. Trop. Dis.* **9**. https://doi.org/10.1371/journal.pntd.0003832.

Branz, A., Levine, M., Lehmann, L., Bastable, A., Ali, S. I., Kadir, K., Yates, T., Bloom, D. & Lantagne, D. 2017 Chlorination of drinking water in emergencies: a review of knowledge to develop recommendations for implementation and research needed. *Waterlines* **36**, 4–39. https://doi.org/10.3362/1756-3488.2017.002.

CAWST 2021 *Aquatabs* [WWW Document]. HWTS Knowledge Base. Available from: https://www.hwts.info/products-technologies/114cf355/aquatabs/technical-information (accessed 22 October 2021).

Centers for Disease Control and Prevention n.d. *Effect of Chlorination on Inactivating Selected Pathogen* [WWW Document]. Safe Water System. Available from: https://www.cdc.gov/safewater/effectiveness-on-pathogens.html (accessed 25 March 2018).

Coates, D. 1985 A comparison of sodium hypochlorite and sodium dichloroisocyanurate products. *J. Hosp. Infect.* **6**, 31–40. https://doi.org/10.1016/s0195-6701(85)80015-3.

Cronin, A. A., Shrestha, D., Cornier, N., Abdalla, F., Ezard, N. & Aramburu, C. 2008 A review of water and sanitation provision in refugee camps in association with selected health and nutrition indicators – the need for integrated service provision. *J. Water Health* **6**, 1–13. https://doi.org/10.2166/wh.2007.019.

D'Mello-Guyett, L., Gallandat, K., Van den Bergh, R., Taylor, D., Bulit, G., Legros, D., Maes, P., Checchi, F. & Cumming, O. 2020 Prevention and control of cholera with household and community water, sanitation and hygiene (WASH) interventions: a scoping review of current international guidelines. *PLoS One* **15**, e0226549. https://doi.org/10.1371/journal.pone.0226549.

Dunoyer, J. n.d. *Lutter Contre le Choléra! Le rôle des secteurs EAH et SMPS dans la lutte contre le choléra. Action contre la Faim-France*.

Finger, F., Bertuzzo, E., Luquero, F. J., Naibei, N., Touré, B., Allan, M., Porten, K., Lessler, J., Rinaldo, A. & Azman, A. S. 2018 The potential impact of case-area targeted interventions in response to cholera outbreaks: a modeling study. *PLoS Med.* **15**, e1002509. https://doi.org/10.1371/journal.pmed.1002509.

Gallandat, K., Stack, D., String, G. & Lantagne, D. 2019 Residual maintenance using sodium hypochlorite, sodium dichloroisocyanurate, and chlorine dioxide in laboratory waters of varying turbidity. *Water* **11**, 1309. https://doi.org/10.3390/w11061309.

Gayer, M., Legros, D., Formenty, P. & Connolly, M. A. 2007 Conflict and emerging infectious diseases. *Emerging Infect. Dis.* **13**, 1625–1631. https://doi.org/10.3201/eid1311.061093.

Global Task Force on Cholera Control 2017 *Ending Cholera: A Global Roadmap to 2030*.

Huq, A., Haley, B. J., Taviani, E., Chen, A., Hasan, N. A. & Colwell, R. R. 2012 Detection, isolation, and identification of *Vibrio cholerae* from the environment. *Curr. Protoc. Microbiol.* **26**, 6A.5.1–6A.5.51. https://doi.org/10.1002/9780471729259.mc06a05s26.

Iqbal, Q., Lubeck-Schricker, M., Wells, E., Wolfe, M. K. & Lantagne, D. 2016 Shelf-life of chlorine solutions recommended in Ebola virus disease response. *PLoS One* **11**, e0156136. https://doi.org/10.1371/journal.pone.0156136.

Johns Hopkins University, International Federation of Red Cross and Red Crescent Societies 2008 *Public Health Guide in Emergencies*, 2nd edn. IFRC, Geneva, Switzerland.

Lamond, E. & Kinyanjui, J. 2012 *Cholera Outbreak Guidelines: Preparedness, Prevention and Control*. Oxfam, Oxford, UK.

Lantagne, D. S. 2008 Sodium hypochlorite dosage for household and emergency water treatment. *Journal AWWA* **100**, 106–119. https://doi.org/10.1002/j.1551-8833.2008.tb09704.x.

Lantagne, D. S. & Clasen, T. F. 2012 Use of household water treatment and safe storage methods in acute emergency response: case study results from Nepal, Indonesia, Kenya, and Haiti. *Environ. Sci. Technol.* **46**, 11352–11360. https://doi.org/10.1021/es301842u.

Morris, J. G., Sztein, M. B., Rice, E. W., Nataro, J. P., Losonsky, G. A., Panigrahi, P., Tacket, C. O. & Johnson, J. A. 1996 *Vibrio cholerae* 01 can assume a chlorine-resistant rugose survival form that is virulent for humans. *J. Infect. Dis.* **174**, 1364–1368. https://doi.org/10.1093/infdis/174.6.1364.

Murphy, J., Cartwright, E., Johnson, B., Ayers, T., Worthington, W. & Mintz, E. D. 2018 An evaluation of a bucket chlorination campaign during a cholera outbreak in rural Cameroon. *Waterlines* **37**, 266–279. https://doi.org/10.3362/1756-3488.00009.

Olson, D., Fesselet, J.-F. & Grouzard, V. 2017 *Management of a Cholera Epidemic*. Médecins Sans Frontières.

Personal communication between authors and laboratory scientists at the US CDC's Waterborne Disease Prevention Branch 2020.

Personal communication between authors and members of the WHO Household Water Treatment Testing Scheme 2017.

Ratnayake, R., Finger, F., Azman, A. S., Lantagne, D., Funk, S., Edmunds, W. J. & Checchi, F. 2021 Highly targeted spatiotemporal interventions against cholera epidemics, 2000–19: a scoping review. *The Lancet Infect. Dis.* **21**, e37–e48. https://doi.org/10.1016/S1473-3099(20)30479-5.

Robertson, R. C. & Pollitzer, R. 1939 Cholera in Central China during 1938 – its epidemiology and control. *Trans. R. Soc. Trop. Med. Hyg.* **33**, 213–232.

Sack, D. A., Sack, R. B., Nair, G. B. & Siddique, A. 2004 Cholera. *The Lancet* **363**, 223–233. https://doi.org/10.1016/S0140-6736(03)15328-7.

Sikder, M., String, G., Kamal, Y., Farrington, M., Rahman, A. S. & Lantagne, D. 2020 Effectiveness of water chlorination programs along the emergency-transition-post-emergency continuum: evaluations of bucket, in-line, and piped water chlorination programs in Cox's Bazar. *Water Res.* **178**, 115854. https://doi.org/10.1016/j.watres.2020.115854.

Spiegel, P. B., Le, P., Ververs, M.-T. & Salama, P. 2007 Occurrence and overlap of natural disasters, complex emergencies and epidemics during the past decade (1995–2004). *Conflict Health* **1**, 2. https://doi.org/10.1186/1752-1505-1-2.

Spina, A., Beversluis, D., Irwin, A., Chen, A., Nassariman, J. N., Ahamat, A., Noh, I., Oosterloo, J., Alfani, P., Sang, S., Lenglet, A. & Taylor, D. L. 2018 Learning from water treatment and hygiene interventions in response to a hepatitis E outbreak in an open setting in Chad. *J. Water Health* **16**, 223–232. https://doi.org/10.2166/wh.2018.258.

UNICEF n.d. *UNICEF Cholera Toolkit (English, French and Arabic)* [WWW Document]. UNICEF. Available from: https://www.unicef.org/cholera/index_71222.html (accessed 20 May 2020).

US EPA, O. 2015 *National Primary Drinking Water Regulations* [WWW Document]. Available from: https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations (accessed 22 October 2021).

Van Den Noortgate, J. & Maes, P. 2010 *Public Health Engineering in Precarious Situations*, 2nd edn. Médecins Sans Frontières, Paris, France.

Wells, E., Wolfe, M. K., Murray, A. & Lantagne, D. 2016 Accuracy, precision, ease-of-use, and cost of methods to test Ebola-relevant chlorine solutions. *PLoS One* **11**, e0152442. https://doi.org/10.1371/journal.pone.0152442.

WHO 2014 *WHO International Scheme to Evaluate Household Water Treatment Technologies: Harmonized Testing Protocol – Technology Non-Specific*.

WHO 2017 *Guidelines for Drinking-Water Quality, 4th Edition, Incorporating the 1st Addendum*, 4th edn. World Health Organization, Geneva, Switzerland.

WHO 2019a *Weekly Epidemiological Record*, Vol. 94, 48, pp. 561–580.

WHO 2019b *Cholera Fact Sheet*.

Wilhelm, N., Kaufmann, A., Blanton, E. & Lantagne, D. 2017 Sodium hypochlorite dosage for household and emergency water treatment: updated recommendations. *J. Water Health* **16**, 112–125. https://doi.org/10.2166/wh.2017.012.

Yates, T., Vujcic, J. A., Joseph, M. L., Gallandat, K. & Lantagne, D. 2018 Water, sanitation, and hygiene interventions in outbreak response: a synthesis of evidence. *Waterlines* **37**, 5–30. https://doi.org/10.3362/1756-3488.17-00015.

First received 18 January 2022; accepted in revised form 18 June 2022. Available online 30 June 2022