

Antibacterial and antiviral effectiveness of two household water treatment devices that use monobrominated hydantoinylated polystyrene

Kyle S. Enger, Emaly S. Leak, Tiong Gim Aw, Angela D. Coulliette and Joan B. Rose

ABSTRACT

Many different household water treatment (HWT) methods have been researched and promoted to mitigate the serious burden of diarrheal disease in developing countries. However, HWT methods using bromine have not been extensively evaluated. Two gravity-fed HWT devices (AquaSure™ and Waterbird™) were used to test the antimicrobial effectiveness of HaloPure® Br beads (monobrominated hydantoinylated polystyrene) that deliver bromine. As water flows over the beads, reactive bromine species are eluted, which inactivate microorganisms. To assess log₁₀ reduction values (LRVs) for *Vibrio cholerae*, *Salmonella enterica* Typhimurium, bacteriophage MS2, human adenovirus 2 (HAdV2), and murine norovirus (MN), these organisms were added to potable water and sewage-contaminated water. These organisms were quantified before and after water treatment by the HWT devices. On average, 6 LRVs against *Vibrio* were attained, as well as 5 LRVs against *Salmonella*, 4 LRVs against MS2, 5 LRVs against HAdV2, and 3 LRVs against MN. Disinfection was similar regardless of whether sewage was present. Polymer beads delivering bromine to drinking water are a potentially effective and useful component of HWT methods in developing countries.

Key words | developing countries, drinking water, MS2, *Salmonella*, sewage, *Vibrio cholerae*

INTRODUCTION

Many different household water treatment (HWT) methods are used by people in underdeveloped regions of the world without access to clean piped drinking water in order to prevent gastroenteritis and diarrhea. Diarrheal disease causes approximately 1.8 million deaths in young children per year in developing countries (Boschi-Pinto *et al.* 2008; World Health Organization 2008), and also interacts with malnutrition to cause widespread morbidity, including poor growth and impaired cognitive development (Guerrant *et al.* 2002). Effective HWT methods include (Sobsey 2002; Schmidt & Cairncross 2009): (1) boiling, which is by far the most common (Rosa & Clasen 2010); (2) various types of filtration,

such as ceramic filters or sand filters; (3) solar disinfection (SODIS), in which water is exposed to sunlight using clear plastic bottles; and (4) chlorine treatment, usually by adding hypochlorite (sometimes with a flocculant) to water. These methods are often combined with safe storage, in which decontaminated water is confined and dispensed (e.g. closed container with a tap) so that it cannot be recontaminated by hands or dippers. However, the effectiveness of HWT can be seriously impacted by inconsistent use (Hunter *et al.* 2009; Enger *et al.* 2013). People in underdeveloped communities must work hard to survive and have difficulty finding time to treat water (Paul 1955); they may also be unable to pay

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seemingly small recurring costs for HWT (Stockman *et al.* 2007). Therefore, a successful HWT technology in poor areas must be simple to use and inexpensive. The effectiveness of a particular HWT technology also depends on the characteristics of the community, and there is no single technology that is best in all settings (Clasen 2009).

Halogen, particularly chlorine, are commonly used in municipal water treatment systems to control microbial contamination in drinking water. It is also a widely promoted HWT method, particularly by adding hypochlorite to drinking water; common examples are aqueous solutions, such as WaterGuard (Lantagne & Gallo 2008), and soluble powders, such as PUR™ or P&G™ flocculant-disinfectant sachets (Chiller *et al.* 2006), which are capable of inactivating $\geq 6 \log_{10}$ of bacteria (Sobsey *et al.* 2008). Bromine is much less commonly used as a water disinfectant than chlorine, but has been used to treat drinking water on US naval vessels for several decades (Dunk 2007). Bromine and chlorine are both strongly oxidizing halogens, and they can both be delivered using N-halamine media (Chen *et al.* 2003), such as HaloPure® beads. These media consist of spherical polymer beads approximately 0.5 mm in diameter, which have a high surface area and can reversibly bind reactive chlorine or bromine species. Although the precise mechanism is unclear, it appears that when microorganisms contact the beads, Cl⁺ or Br⁺ is transferred to microorganisms, oxidizing various targets on the microorganism and causing inactivation (Chen *et al.* 2003). In this way, disinfectant is released in response to demand from the water, ensuring an appropriate level of disinfection without the user having to determine the proper dose (Chen *et al.* 2003). Under certain circumstances, brominated N-halamine media have been found to have greater antimicrobial activity than chlorinated (Panangala *et al.* 1997; McLennan *et al.* 2009; Coulliette *et al.* 2010).

HaloPure® beads are the active component of several HWT devices which have been produced, sold, and used in developing countries (Williams & Bridges 2010). Two examples are AquaSure™ and Waterbird™, both of which have an upper tank in which water is placed; by gravity, the water flows through a canister containing the HaloPure® beads. Once water has passed through the canister, it collects in a lower tank with a spigot for storing and dispensing disinfected water. Since the water contains residual halogen, additional disinfection could occur while

the water remains in the lower tank. The AquaSure™ device was previously sold in India by Eureka Forbes Ltd, while the WaterBird™ device was under development by HaloSource, Inc. at the time of this study.

Prior work in our laboratory has also evaluated chlorinated HaloPure® beads against *V. cholerae* and *S. enterica* Typhimurium (Coulliette *et al.* 2013), as well as MS2 using chlorinated and brominated HaloPure® beads (Coulliette *et al.* 2010). Another study in our laboratory (McLennan *et al.* 2009) examined both brominated and chlorinated HaloPure® beads against fecal indicator bacteria, heterotrophs, and coliphage from sewage, and indicated increased activity against coliphage for bromine compared to chlorine. These studies all used the AquaSure™ device.

The goal of this study was to evaluate the ability of monobrominated HaloPure® N-halamine beads (monobrominated hydantoinylated polystyrene, HaloSource, Inc., Bothell, Washington, USA, USEPA registration number 72083-3) to inactivate *Vibrio cholerae*, *Salmonella enterica* Typhimurium, bacteriophage MS2, human adenovirus 2 (HAdV2), and murine norovirus (MN). While MS2 is commonly used as a surrogate for human enteric viruses, various species and types of *Vibrio*, *Salmonella*, adenovirus, and norovirus are human enteric pathogens, particularly in developing countries. Measurements of antimicrobial activity against such organisms may be more useful for developing country risk assessments than activity against conventional indicator organisms.

METHODS

Nature of the HWT devices tested

This study used modified mass-produced AquaSure™ devices (Eureka Forbes Ltd, Mumbai, India; Figure 1(a)) and prototype Waterbird™ devices (HaloSource, Inc., Bothell, Washington, USA; Figure 1(c)), both of which used HaloPure® Br beads (HaloSource, Inc., Bothell, Washington, USA). Both devices consist of an upper tank for untreated water and a lower tank for treated water. By gravity, water flows from the upper tank through a canister containing the HaloPure® Br beads, and treated water drips into the lower tank, where it can be dispensed by a spigot. Within the AquaSure™ canister, water follows a tortuous

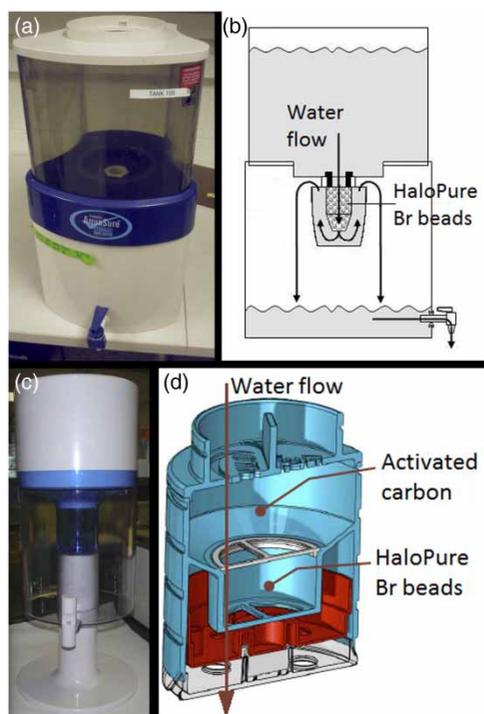


Figure 1 | AquaSure™ (left) and Waterbird™ (right) devices. Both are gravity-fed, with an upper tank for untreated water and a lower tank with a spigot for treated water. (a) AquaSure™ photo; (b) AquaSure™ diagram; (c) Waterbird™ photo; (d) Waterbird™ cartridge, found in the center of the device between the upper and lower tanks (diagram provided by HaloSource, Inc.).

path to restrict its flow (Figure 1(b)), while in the Waterbird™ water flows directly downward (Figure 1(d)), with its flow restricted primarily by a prefilter. Unmodified AquaSure™ devices use a prefilter to remove particles from the

influent water before treatment by HaloPure® Cl (chlorinated) beads, as well as activated charcoal to remove organic compounds. These parts of the unit were removed, and the beads replaced, in order to isolate the disinfection effect of the HaloPure® Br beads and to conservatively estimate their antimicrobial activity. The Waterbird™ device was unmodified; water flowed downward through a ceramic prefilter followed by activated charcoal before encountering the HaloPure® Br beads.

Experimental design

All experiments followed a similar pattern; the number of experiments for each device and microorganism are listed in Table 1. At least 5 L of clean water was passed through the devices before the experiment began. The spigot on the lower tank of the device was left open at all times. A timer was started when the influent containing microorganisms was poured into the upper tank. When water began flowing through the spigot, this was termed ‘first flush’; the time and flow rate were recorded, and samples were taken for determination of the total bromine residual and for microbial assays. Two or three additional samples at later time points were also analyzed. Total bromine residuals were measured colorimetrically (Hach chlorine test kit model CN-66, Hach Co., Loveland, Colorado, USA), using the total chlorine reagent (DPD method), adjusted for the molecular weight of bromine by multiplying the

Table 1 | Summary of conservatively estimated log₁₀ reduction values (LRVs) for each organism tested^a

AquaSure device organism	n	LRVs at first flush (worst case)			LRVs at 45 min. (best case)		
		Mean	95% CI ^b	Minimum ^c	Mean	95% CI ^b	Minimum ^c
<i>Vibrio</i>	8	6.0	5.4 to 6.6	5.7	6.1	5.6 to 6.7	5.7
<i>Salmonella</i>	7	4.6	3.5 to 5.6	3.2	6.6	6.3 to 6.9	6.8
Bacteriophage MS2	11	3.9	3.1 to 4.7	2.0	5.4	4.7 to 6.1	4.5
Human adenovirus 2	6	4.9	4.5 to 5.2	5.6	4.9	4.5 to 5.2	5.6
Waterbird device organism	n	LRVs at 15 min. (worst case)			LRVs at first flush (best case)		
		Mean	95% CI ^b	Minimum ^c	Mean	95% CI ^b	Minimum ^c
Bacteriophage MS2	36	3.9	3.3 to 4.4	1.5	6.4	6.0 to 6.7	3.8
Murine norovirus	24	3.1	2.7 to 3.5	NA	3.0	2.6 to 3.3	NA

^aCombines experiments with and without sewage. Nondetects were treated as if 3 CFU, 3 PFU, or 0.94 MPN were detected, depending on the organism.

^b95% confidence interval of the mean, which included nondetects.

^cMinimum LRV directly measured, excluding nondetects. All MN measurements were nondetects, so those LRVs are likely to be substantially underestimated.

concentration by 2.25. It could detect a minimum of approximately 0.1 mg/L of total bromine. Any bromine residual in the samples for microbial assays was immediately quenched by adding sodium thiosulfate solution (final concentration of approximately 0.1% m/v).

Experiments with the AquaSure™ and Waterbird™ devices differed in several ways. Clean water was passed through the AquaSure™ immediately before the experiment, while clean water was passed through the Waterbird™ the evening before the experiment. Samples from the AquaSure™ were taken at first flush, 15 minutes, and 45 minutes. Since flow was slower through the Waterbird™ device, sampling occurred at first flush, 15 minutes, 45 minutes, and 120 minutes. Ten liters of influent were used with the AquaSure™ device, while 5 L were used with the Waterbird™. AquaSure™ experiments used various mixtures of *Vibrio*, *Salmonella*, MS2, and adenovirus; all Waterbird™ experiments used MS2, and some of those experiments also included MN. The pH of the influent in the AquaSure™ experiments was not adjusted (pH approximately 7.8), while the influent for the Waterbird™ experiments was adjusted to pH 7.5 or 9.0 using HCl or NaOH solutions in an attempt to investigate the effect of pH on microbial inactivation. The AquaSure™ experiments preceded the Waterbird™ experiments and were performed by different investigators (primarily K. S. Enger for AquaSure™, and primarily E. S. Leak for Waterbird™). The experiments were intended to estimate the inactivation of various microorganisms by HaloPure® Br beads, rather than to compare inactivation between the two devices.

The AquaSure™ experiments contained microorganisms as follows: 10 mL of *Salmonella enterica* Typhimurium LT2 (HER 1023; Centre de référence pour virus bactériens Félix d'Hérelle, Université Laval, Québec, Canada) grown and suspended in Rappaport-Vassiliadis R10 broth (Becton, Dickinson & Co., Sparks, Maryland, USA, reference number 218581), 10 mL of *Vibrio cholerae* (ATCC 51395, American Type Culture Collection, Manassas, Virginia, USA) grown and suspended in trypticase soy broth (TSB; Becton, Dickinson & Co., Sparks, Maryland, USA, ref. number 211768), 10 mL of HAdV2 (ATCC VR-846) suspension in minimal essential medium (MEM; Cellgro, Mediatech, Inc., Manassas, Virginia, ref. number 10-010-CV) with 2% fetal bovine serum (FBS; Atlanta Biologicals,

Lawrenceville, Georgia, ref. number S11050H), and bacteriophage MS2 suspended in 10 mL TSB. The Waterbird™ experiments used similarly prepared MS2, as well as MN (uncharacterized strain from Dr Kellogg Schwab at Johns Hopkins University) suspension in phosphate buffered saline (PBS).

Although AquaSure™ experiments using *Salmonella* and *Vibrio* also included MS2 and HAdV2, they showed low log₁₀ reduction values (LRVs) of about 1 for both viruses (data not shown), in contrast with previously reported LRVs of 5 for MS2 (Coulliette *et al.* 2010). Further experiments indicated that the MEM in which the HAdV2 was suspended appeared to protect HAdV2 and MS2 from inactivation (data not shown). Therefore, additional experiments with HAdV2 and MS2 were conducted, using purified HAdV2 (method described below). The influent for those experiments included 2 mL of bacteriophage MS2 suspension in TSB and 3 mL of HAdV2 suspension in PBS, mixed with well water and optionally 500 mL of raw sewage, for a total influent volume of 10 L.

The microorganisms were added to nonchlorinated potable well water (Williamston, Michigan, USA; other characteristics described elsewhere (Coulliette *et al.* 2010)) to make the influent. Some experiments also included 5% (v/v) raw sewage from the East Lansing waste water treatment plant (East Lansing, Michigan, USA). Across all experiments, the concentrations of pathogens in the influent were 6×10^5 to 1×10^6 colony-forming units (CFU)/mL for *V. cholerae*, 8×10^4 to 2×10^5 CFU/mL for *S. enterica*, 2×10^4 to 2×10^5 most probable number (MPN)/mL for HAdV2, 7×10^4 to 2×10^8 plaque-forming units (PFU)/mL for MS2, and 1×10^2 to 1×10^5 PFU/mL for MN.

Growth of viruses

MS2 was grown and quantified as previously described (Coulliette *et al.* 2010). Briefly, water samples were diluted in phosphate-buffered water (PBW; pH 7.2) and aliquots were mixed with melted trypticase soy agar (TSA; Becton, Dickinson & Co., Sparks, Maryland, USA, reference number 211043) and a suspension of *Escherichia coli* Famp (ATCC 700891), and overlaid onto TSA plates. After incubation at 37 °C for approximately 20 hours, plaques were counted, quantifying MS2 in PFU/mL.

To obtain HAdV2 suspension, viruses were grown using the A549 cell line (ATCC CCL-185; originally from a human lung tumor). Monolayers were grown by incubating in 28 mL of MEM (Cellgro, Mediatech, Inc., Manassas, Virginia, ref. number 10-010-CV) with 2% FBS (Atlanta Biologicals, Lawrenceville, Georgia, reference number S11050H) in 75 cm² tissue culture flasks in a 5% CO₂ atmosphere. To produce concentrated virus, it was inoculated onto an 80–95% confluent monolayer. Once 90% of the monolayer was destroyed, cells were frozen, thawed, and agitated three times. The resulting suspension was filtered through 0.45 and 0.22 micron filters (Millipore, Billerica, Maryland, ref. numbers SLHA033SS and SLGV03RSS). The filtered suspension was then ultrafiltered (Amicon Ultra-15 100 kilodalton, Millipore, Billerica, Massachusetts, reference number UFC910024), washed three times with PBS, and stored in PBS at –80 °C. It was thawed by hand before adding to the influent water. It contained 2.4×10^{10} MPN/mL by the three-replicate MPN technique (de Man 1983).

To obtain MN suspension, RAW 264.7 cells (ATCC TIB-71; mouse macrophage cells) were grown on maintenance media (high-glucose DMEM (Hyclone, Logan, Utah, #SH30243.02) with 2% low-endotoxin FBS (Hyclone, Logan, Utah, #SH30070.03), 1% HEPES free acid (Amarasco, Solon, Ohio, #J848), 1% MEM NEAA (Lonza, Walkersville, Maryland, #13-114E), 1% penicillin/streptomycin, and 1% L-glutamine) in 150 cm² flasks until they were >80% confluent. They were then infected with 6 mL of diluted virus stock in maintenance media, such that there were no more virus particles than cells in the flasks. Infected flasks were incubated for 1 hour at 37 °C and 5% CO₂, and were rocked every 15 minutes. After 1 hour of incubation, the inoculum was removed from the flasks and complete DMEM without FBS was added. Flasks were incubated for 2–7 days at 37 °C and 5% CO₂ until they showed cytopathic effect (CPE). The flasks were then frozen at –80 °C overnight and subsequently thawed at room temperature; this was repeated three times. The resulting cell/virus suspension was filtered through a 0.45 and 0.22 micron filter (Millipore) and then frozen at –80 °C. It was subsequently ultrafiltered and washed in the same way as HAdV2 to produce a purified virus suspension in PBS for the experiments. The

purified stock contained approximately 1×10^7 PFU/mL and was frozen at –80 °C.

Quantification of microorganisms

V. cholerae and *S. enterica* Typhimurium bacteria were enumerated by serially diluting in PBW, followed by membrane filtration. Membranes (GN-6 MetriCel[®] grid, 0.45 µm pore size, Pall Corp., Ann Arbor, Michigan, USA) were placed onto thiosulfate citrate bile salts sucrose agar (TCBS; Becton, Dickinson & Co., Sparks, Maryland, USA, reference number 265020) and xylose lysine deoxycholate agar (XLD; Becton, Dickinson & Co., Sparks, Maryland, USA, reference number 278850) plates, respectively, and incubated at 37 °C for approximately 20 hours. Colonies were then counted, quantifying the bacteria as CFU/mL.

For HAdV2, water samples were frozen at –80 °C until they could be assayed. They were then thawed and serially diluted in PBS. A549 cell monolayers were prepared in 25 cm² tissue culture flasks containing 8 mL of medium. The medium was discarded, and monolayers were rinsed with 8 mL PBS. Diluted samples (2 mL volume) were added to the flasks, which were incubated at 37 °C for 60 minutes, being rocked manually every 15 minutes. The sample was poured out of the flask, 8 mL of MEM with 2% FBS was added, and incubated as described above. Monolayers were examined microscopically over 7 days to detect CPE, compared with a negative control. Monolayers with no evidence of CPE received fresh MEM with 2% FBS and were incubated for another 7 days before being considered negative. Evidence of CPE (i.e. cell destruction) at any time was considered a positive result for purposes of three-replicate MPN calculation.

For MN, water samples were frozen at –80 °C until they could be assayed. Confluent flasks of RAW 264.7 host cells were split to make 6-well 9.6 cm² cell culture plates (Corning, Corning, New York, #3516); approximately 3×10^6 cells were added to each well. The plates were incubated for 24 hours in a 37 °C 5% CO₂ incubator. The growth media was then removed from each well and wells were inoculated with 1:10 dilutions (in maintenance media) of the samples, using 0.5 mL inoculum per well. Duplicates were processed of each sample, as well as positive and negative controls. The plates were then incubated and rocked for 1 hour at room temperature (~20 °C). The overlay media had two

components: (1) 1.5% agarose (1.5 g agarose (Cambrex, Rockland, Maine, #50111) dissolved in 50 mL NanoPure water, autoclaved for 15 minutes, and brought to 48 °C); and (2) 2×MEM (2×MEM (Sigma, St Louis, Missouri, #M3024) with 10% low-endotoxin FBS, 2% L-glutamine, 2% penicillin/streptomycin, and 1% HEPES) brought to 37 °C. Immediately before use, equal parts of the agarose and 2×MEM solutions were combined and thoroughly mixed. Two mL were added to each well, and it was allowed to solidify at room temperature (~20 °C) for 30 minutes. The plates were then incubated at 37 °C and 5% CO₂ for 24 hours. Subsequently, 2 mL of fresh overlay media (with 2% neutral red (Sigma, St Louis, Missouri, #N2889)) were added on top of the first overlay in each well to stain the living cells and visualize the plaques. The plates solidified at room temperature (~20 °C) and were incubated for 24 hours at 37 °C and 5% CO₂. After 24 and 48 hours, plaques were counted to obtain a measurement of PFU per mL of sample.

Statistical analysis

Microorganisms were sometimes undetectable after treatment. To conservatively estimate the LRV, bacterial, MS2, and MN nondetects were considered to have 3 CFU or PFU in the least dilute specimen measured. For example, if 1 mL of undiluted sample was plated for *Vibrio*, and no CFUs were observed, the concentration would be considered to be 3 CFU/mL. This method ensures that overestimation of LRVs is rare, since a Poisson distribution whose mean is 3 returns a zero value 5% of the time. If HAdV2 was not detected by MPN, the upper limit of the 95% confidence interval was used (i.e. if all three tubes were negative, the 95% confidence interval would be 0–0.94 MPN, and the measurement would be considered 0.94 MPN). Statistical analysis and charting of data were carried out using R version 3.1 (R Foundation for Statistical Computing, Vienna, Austria). Repeated measures ANOVA (analysis of variance) was used to simultaneously determine whether presence or absence of sewage was associated with the mean value or the time trend of several dependent variables: (1) flow rate, (2) bromine residual, (3) mean LRVs for *Vibrio*, *Salmonella*, MS2, and HAdV2. Measurements of the untreated influent water were not included in the ANOVA analyses. An α value of 0.05 was used for significance testing. Nine Waterbird™

experiments lacked data at the 15 minute time point because ‘first flush’ occurred later than 15 minutes; to account for this, repeated measures ANOVA was run in three ways: (1) using all experiments, but omitting all measurements at 15 minutes; (2) using only experiments that had data for all time points; (3) using all data from all experiments, but imputing the median for the missing data at 15 minutes.

RESULTS

Flow rate and bromine residual from the devices

Although the flow rate through both devices decreased with time, the AquaSure™ device had faster flow than the Waterbird™ (Figure 2). There was a significant effect of time on total bromine residual for both devices (Figure 3); however, the residual was highly variable, ranging from 0.11 to 1.69 mg/L of total bromine for AquaSure™, and from 0.023 to 2.93 mg/L for Waterbird™. Neither flow rate nor total bromine residual differed by presence or absence of sewage (Figures 2 and 3), although the effect of sewage on the total bromine residual with the AquaSure™ device approached statistical significance ($P = 0.08$).

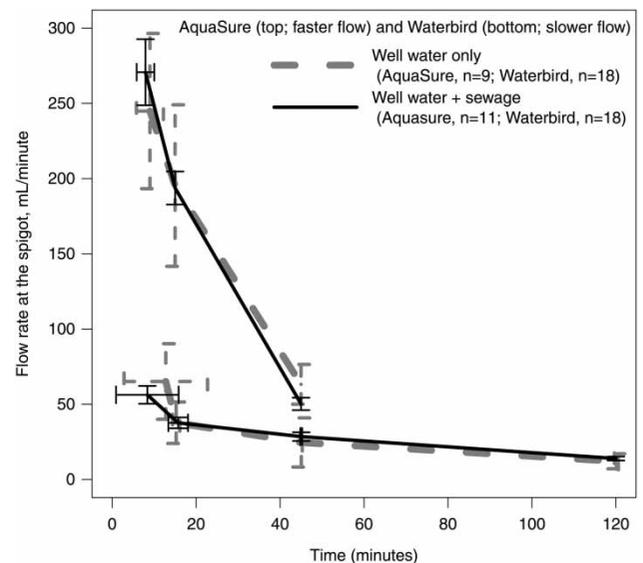


Figure 2 | Flow rate (mL/minute) from the spigots of the AquaSure™ (top) and Waterbird™ (bottom) devices. Error bars are ± 1 standard deviation. There is a significant time trend for both devices ($P < 2 \times 10^{-16}$). Flow rates are similar regardless of whether sewage was used.

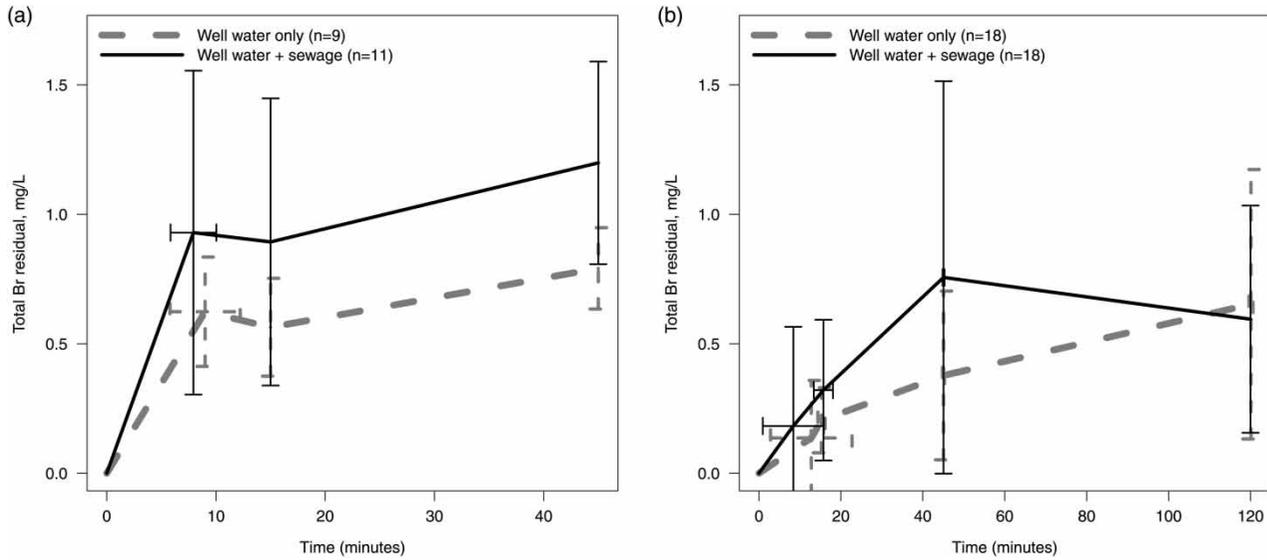


Figure 3 | Total bromine residual (mg/L free and combined bromine) delivered by (a) AquaSure™ and (b) Waterbird™ devices. Error bars are ±1 standard deviation. There is a significant effect of time on residual for both devices ($P < 9 \times 10^{-5}$). There is weak evidence for differing AquaSure™ residual by presence of sewage ($P = 0.08$), but no evidence for differing Waterbird™ residual by presence of sewage.

Inactivation of microorganisms (AquaSure™)

On average at first flush, regardless of whether sewage was used, LRVs of 6.0 were attained for *Vibrio*, 4.6 LRVs for *Salmonella*, 3.9 LRVs for MS2, and 4.9 LRVs for HAdV2 (Table 1). In all cases, at least 99% (two LRVs) of each

microorganism was inactivated (Table 1). Measurements of *Vibrio* were usually near the detection limit (Figure 4), so it was not possible to see whether *Vibrio* inactivation changed with time. However, *Salmonella* were inactivated more effectively at 45 minutes than at first flush (Figure 5), and

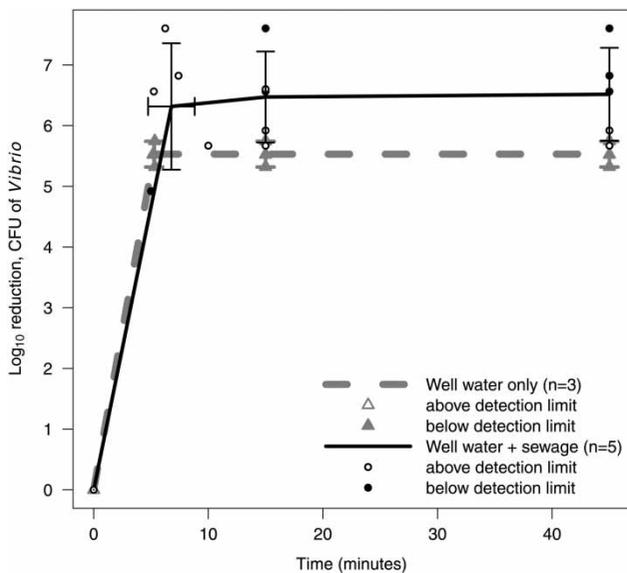


Figure 4 | LRV of *Vibrio cholerae* in the AquaSure™ device, CFU. Error bars are ±1 standard deviation. There was no evidence of differing LRVs by presence of sewage.

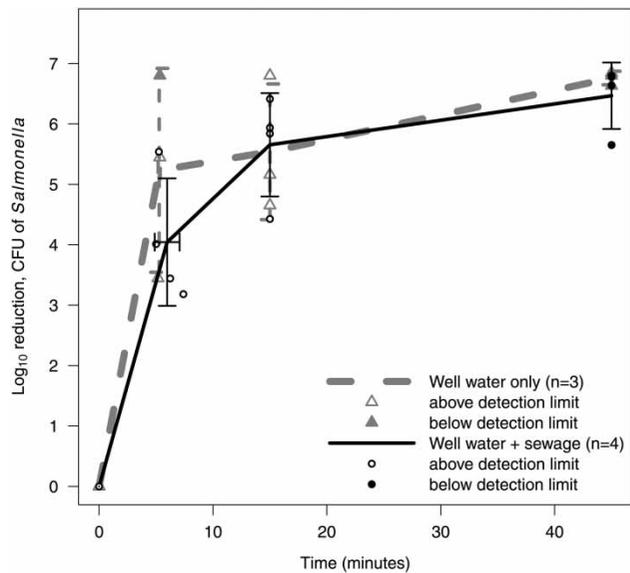


Figure 5 | LRV of *Salmonella enterica* Typhimurium in the AquaSure™ device, CFU. Error bars are ±1 standard deviation. There was no evidence of differing LRVs by presence of sewage, although there was a significant time trend overall ($P = 0.004$).

MS2 showed a similar trend (Figure 6(a)). However, it is unclear whether inactivation of HAdV2 increased with time, because (as for *Vibrio*) the measurements were near the detection limit. The trend toward improved inactivation of *Salmonella* and MS2 from first flush to 45 minutes is consistent with slower flow rates at 45 minutes (Figure 2) and correspondingly longer contact time with the beads. There was no evidence that the presence or absence of sewage affected inactivation of any tested organism (Figures 4–7).

Inactivation of microorganisms (Waterbird™)

In contrast to the AquaSure™ device, inactivation of MS2 was highest at first flush (Figure 6(b) and Table 1; mean LRV of 6.4) and lowest at 15 minutes (Figure 6(b) and Table 1; mean LRV of 3.9). Only MS2 was tested in both the AquaSure™ and Waterbird™ devices. The Waterbird™ device also attained LRVs of 3.0 for MN on average (Table 1); however, all post-treatment measurements of MN detected no viruses, so the LRVs are effectively determined by the concentration of MN in the influent. Fifteen of the 24 experiments with MN were able to detect LRVs >3.0; the maximum LRV detectable by any MN experiment was 4.2. The three statistical methods to account for the nine missing data points at 15 minutes all yielded similar results.

These data points were missing because first flush occurred later than 15 minutes in those experiments. Inactivation was similar regardless of pH.

DISCUSSION

The HaloPure® Br beads were effective against *Salmonella* and *Vibrio* bacteria, bacteriophage MS2, HAdV2, and MN. Although both devices commonly inactivated >4 log₁₀ of MS2, the LRVs were highly variable (Figure 6), and LRVs ≤2.0 were sometimes observed. Likely sources of LRV variation are natural variation in source water and sewage chemistry, temperature of the water, variation among lots of HaloPure® beads, and effects from cultivation and storage of different batches of microorganisms. Although it would be desirable to increase inactivation and reduce its variation, highly consistent use of HWT appears more important than high HWT LRVs for reducing the risk of waterborne disease (Enger et al. 2013).

The devices inactivated microorganisms similarly whether potable water or sewage-contaminated water was used. Although it is unclear exactly how long-term usage with sewage-contaminated water would have affected the

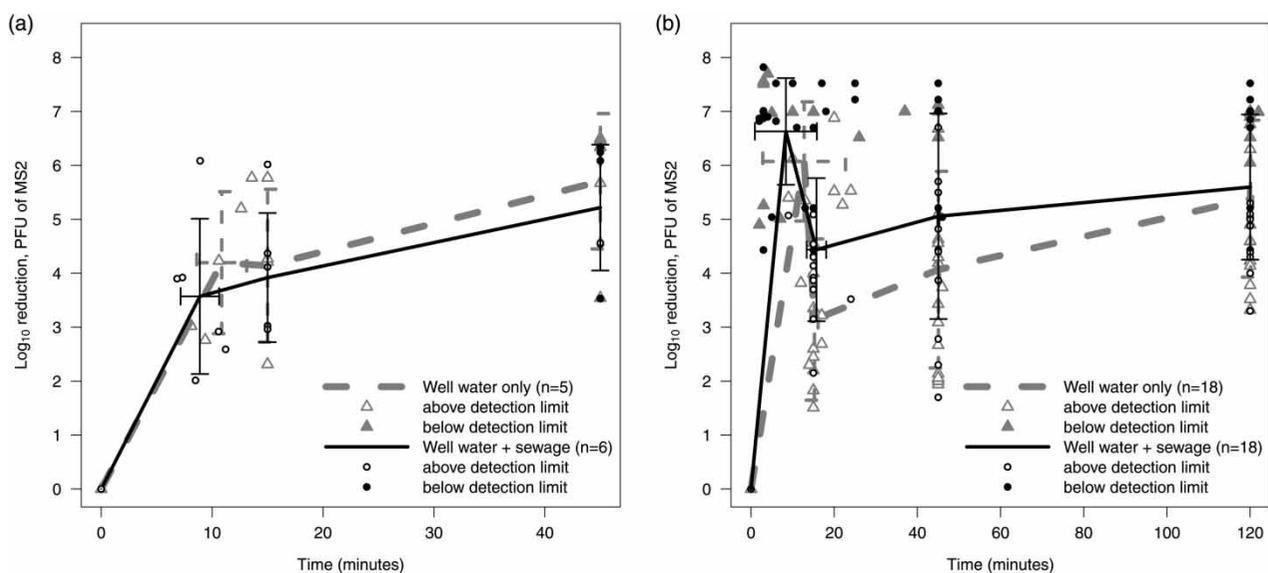


Figure 6 | LRV of bacteriophage MS2, PFU in the (a) AquaSure™ and (b) Waterbird™ devices. Error bars are ±1 standard deviation. There is a significant effect of time on LRV for both devices ($P < 10^{-5}$). Although there was no evidence of differing LRVs by presence of sewage with the AquaSure™ device, there was weak evidence of differing LRVs by presence of sewage with the Waterbird™ device ($P = 0.03$ – 0.14 , depending on how missing data were handled).

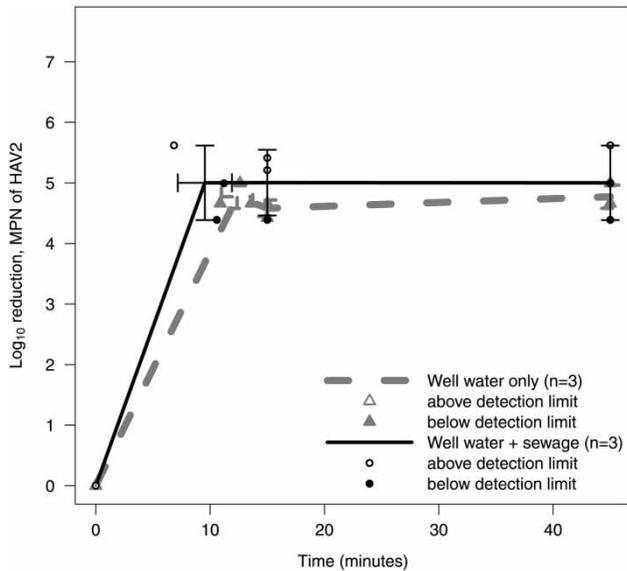


Figure 7 | LRV of HAdV2 in the AquaSure™ device, MPN. Error bars are ± 1 standard deviation. There was no evidence of differing LRVs by presence of sewage.

performance of the beads, increased bromine demand from the sewage would deplete bromine more quickly from the beads.

The two devices behaved quite differently regarding flow. The AquaSure™ device had a faster flow rate and LRVs for MS2 and *Salmonella* tended to increase as the top tank gradually emptied and the flow rate slowed. In contrast, the Waterbird™ device inactivated more MS2 at first flush, with a temporary decrease in inactivation at 15 minutes. A possible explanation is that the AquaSure™ was flushed with clean tap water immediately before the experiment, while the Waterbird™ was flushed the evening before. Bromine dissolved in the moisture between the beads in the AquaSure™ would be flushed out just before the experiment, while the Waterbird™ could have eluted bromine overnight into the moisture between the beads, where it would be immediately available to inactivate microorganisms at first flush, and lead to higher LRVs.

The LRVs reported here are conservative, for several reasons. If no microorganisms were detected in a sample, it was analyzed as if a number of organisms slightly higher than the detection limit had been detected. Organisms were also frequently undetectable in treated water, so use of higher concentrations of organisms in the influent would yield higher LRV estimates, particularly for MN. The devices tested might also perform more poorly than complete, mass-produced versions. For example, the AquaSure™ device was modified to

remove its prefilter, which would probably reduce its efficacy. Also, the Waterbird™ device was a prototype, and the mass-produced version might behave somewhat differently. Furthermore, the residual bromine that was detected in all treated water samples could continue to inactivate microorganisms in stored treated water over time. Such inactivation would be particularly important if contaminated water was poured into the upper tank in the evening, with treated water being dispensed from the lower tank in the morning. It would be less important if water was consumed immediately after being treated.

Previous work with HaloPure® Br beads in the AquaSure™ device in our laboratory using MS2 showed LRVs of 4–5 at first flush and 45 minutes, LRVs of 5–7 at 90 minutes (Coulliette *et al.* 2010), which are consistent with the results of this study. Similar experiments using HaloPure® Cl beads showed lower LRVs of 2–3 at first flush and 45 minutes, but LRVs of 4 at 90 minutes; however, one HaloPure® Cl experiment showed much higher MS2 LRVs (up to 10 at 90 minutes), perhaps due to variability in manufacturing (Coulliette *et al.* 2010). Previous work with HaloPure® Cl beads in the AquaSure™ device in our laboratory using *S. typhimurium* and *V. cholerae* showed LRVs of about 6 for *S. Typhimurium* and 6–7 for *V. cholerae*, indicating similar activity against bacteria by HaloPure® Br and Cl beads. Another inactivation study in our laboratory of naturally occurring fecal indicator and heterotrophic bacteria in sewage gave similar LRVs for HaloPure® Br and Cl beads: LRVs of 3 (*Clostridium* spp.) to 6 (total coliforms) depending on the type of bacteria examined (McLennan *et al.* 2009). That study also indicated LRVs of 1 against naturally occurring coliphage by HaloPure® Cl, but LRVs of >2 by HaloPure® Br (all such samples were below the detection limit for coliphage, yielding a conservative estimate).

The HaloPure® Br beads are potentially useful for HWT in developing countries. The ability of the beads to release more halogen in response to increased demand allows disinfection to be maintained in sewage-contaminated water with high halogen demand. The beads also simplify HWT since the user does not need to measure or mix doses of disinfectant, or adjust dosage depending on halogen demand. Although the user must still purchase and replace the canisters that contain the beads in order to maintain disinfection, brominated beads require less frequent replacement than chlorinated beads (Williams, J. F., HaloSource Inc., personal communication).

A variety of HWT devices using cartridges containing HaloPure[®] Br beads are being sold in developing countries; this includes gravity-fed units like those tested here (treating ~1,500 L per cartridge), and water pitcher units (treating ~320 L per cartridge). Cartridges are simple to replace, assuming they are available in local markets. Although this form of maintenance will remain a barrier to long-term compliance, all HWT methods require some maintenance. Perception of unpleasant tastes or odors appear less likely with bromine than chlorine, but such perceptions vary by: water characteristics; cultural background; experience with tastes/odors; and other factors (McDonald *et al.* 2009; Coulliette *et al.* 2010). Brominated beads also appear to have greater antiviral activity than chlorinated beads (Panangala *et al.* 1997; McLennan *et al.* 2009; Coulliette *et al.* 2010), although antibacterial activity appears similar (Sun *et al.* 1995; McLennan *et al.* 2009; Coulliette *et al.* 2010).

Toxic or carcinogenic disinfection byproducts (DBPs) are a potential drawback of bromine and chlorine HWT. This is a complicated subject beyond the scope of this paper; although DBP production can differ greatly depending on treatment and source water characteristics, contaminated waters with large amounts of organic material are likely to enhance production of DBPs (Heeb *et al.* 2014). However, DBP risks should be considered in the context of serious and widespread risks from diarrheal disease, which causes approximately 1.8 million deaths in young children per year in developing countries (Boschi-Pinto *et al.* 2008; World Health Organization 2008), and also interacts with malnutrition to cause widespread morbidity, including poor growth and impaired cognitive development (Guerrant *et al.* 2002). WHO drinking water guidelines prioritize effective disinfection over DBP risks (World Health Organization 2011), and HaloPure[®] Br beads have been certified under NSF/ANSI standard 42, indicating they do not produce excessive amounts of DBPs in test water (National Sanitation Foundation International 2014). Nonetheless, DBPs and LRVs should be evaluated for these devices under conditions that mimic household use in developing countries (contaminated source waters, high temperatures, long-term use, etc.).

The total bromine residual in the treated water samples was highly variable (Figure 3). The precise meaning of the value of the bromine residual is unclear. We were unable to distinguish free bromine from combined bromine; free bromine would be more reactive, and consequently a more

effective disinfectant. Furthermore, since the HaloPure[®] beads release bromine on demand when they are in contact with contaminated water, it is difficult to determine contact time, which may be loosely related or unrelated to the total bromine residual in treated water. The main significance of the bromine residual measurements is qualitative verification that bromine was released into the water by the beads.

CONCLUSIONS

HaloPure[®] Br beads can effectively inactivate bacteria and viruses in contaminated water in the laboratory. The conservatively estimated LRVs reported here often, but not always, exceed the United States Environmental Protection Agency guidelines for microbiological purifiers of 6 LRVs for bacteria and 4 LRVs for viruses (USEPA 1987). The effectiveness of HaloPure[®] Br remains unclear against protozoan pathogens (such as *Cryptosporidium* or *Giardia*) or other microorganisms that have not been directly tested. HaloPure[®] Br has also shown >4 LRVs against rotavirus (Panangala *et al.* 1997), and 3 to >6 LRVs against a wide variety of bacteria (Sun *et al.* 1995; Chen *et al.* 2003; McLennan *et al.* 2009). However, LRVs attainable by HaloPure[®] Br HWT devices in the field will depend on design characteristics of the device and characteristics of the locally available source water, and the ultimate effectiveness of any type of HWT depends upon households using it correctly and consistently. HWT devices using HaloPure[®] Br should be evaluated in developing country communities for efficacy, safety, acceptability, and consistency of use.

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CONFLICT OF INTEREST STATEMENT

This work was supported by a grant from HaloSource, Inc., makers of HaloPure[®] beads. Although this represents a potential conflict of interest, we attest that HaloSource,

Inc. did not influence the design of the study, the results, or the decision to publish the results.

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