Mechanisms of post-supply contamination of drinking water in Bagamoyo, Tanzania

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ABSTRACT

Access to household water connections remains low in sub-Saharan Africa, representing a public health concern. Previous studies have shown water stored in the home to be more contaminated than water at the source; however, the mechanisms of post-supply contamination remain unclear. Using water quality measurements and structured observations of households in Bagamoyo, Tanzania, this study elucidates the causal mechanisms of the microbial contamination of drinking water after collection from a communal water source. The study identifies statistically significant loadings of fecal indicator bacteria (FIB) occurring immediately after filling the storage container at the source and after extraction of the water from the container in the home. Statistically significant loadings of FIB also occur with various water extraction methods, including decanting from the container and use of a cup or ladle. Additionally, pathogenic genes of *Escherichia coli* were detected in stored drinking water but not in the source from which it was collected, highlighting the potential health risks of post-supply contamination. The results of the study confirm that storage containers and extraction utensils introduce microbial contamination into stored drinking water, and suggest that further research is needed to identify methods of water extraction that prevent microbial contamination of drinking water.

Key words | diarrheal pathogens, drinking water quality, fecal bacteria, recontamination, Tanzania, waterborne disease

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INTRODUCTION

Water-, sanitation-, and hygiene-related (WASH) diseases account for a major share of the global disease burden, particularly among children (Prüss *et al.* 2002). Approximately 1.3 million children under 5 years of age die each year due to diarrheal illness (Black *et al.* 2010). People without access to piped water connections in the home are disproportionately affected by these illnesses (Howard & Bartram 2003). Nonetheless, the Joint Monitoring Program's classification of 'access to improved drinking water' includes access to communal water sources such as public taps and wells (WHO 2010). The majority of the investments to improve water access in sub-Saharan Africa during the period 1990–2010 have been in the form of improved communal water infrastructure (WHO 2010). As a result, coverage with household water connections remains low

in sub-Saharan Africa. With only 16% of the population in Sub-Saharan Africa having private connections, most households must travel to a water source to collect drinking water and consequently store the water in the home (WHO 2010).

The limited health benefits of improved communal water infrastructure have been attributed to several different causes. First, researchers have found that the farther a water source is from a household, the quantity of water that the household uses for hygiene purposes *per capita* decreases (Prost & Négrel 1989; Curtis *et al.* 1995) and this, in turn, can increase the transmission of enteric disease (Cairncross & Valdmanis 2006). Additionally, associations between the time burden of fetching and child welfare have been found, suggesting increased time collecting water leaves less time for child care and domestic chores (Koolwal &

Van de Walle 2010). A recent cross-sectional study by Pickering & Davis (2012) found a positive association between water source walk time and child health. Finally, many studies have found that the microbial quality of water stored in the home deteriorates with time, which represents a public health concern (Wright *et al.* 2004).

Several studies have demonstrated that drinking water can be re-contaminated during storage in the home (Wright *et al.* 2004; Levy *et al.* 2008; Pickering *et al.* 2010). It is notable that in-home storage is common not just for households using shared point sources, but also for those with individual water connections if supply is intermittent and/or the tap is located in the yard or compound, rather than in the home. Levy *et al.* (2008) demonstrated that drinking water collected from unimproved surface water sources was re-contaminated by comparing water quality in storage containers that were accessed by the household and control, isolated, storage containers. Pickering *et al.* (2010) showed that, in Tanzania, drinking water from improved water sources that had been stored in the home was significantly more contaminated than the sources from which it was collected. Re-contamination of drinking water in the home represents a significant health risk, particularly if consumers assume the water to be clean because it was collected from an improved source (Trevett *et al.* 2005b).

The most significant modes of re-contamination of stored drinking water remain unclear. To the authors' knowledge, none of the studies referenced above provide strong evidence regarding the causal mechanisms of re-contamination. Several studies suggest that contaminated hands entering stored water could be a major contamination source (Pinfold 1990; Roberts *et al.* 2001; Trevett *et al.* 2005a, 2005b; Pickering *et al.* 2010). The storage or collection container, as well as water extraction utensils, have also been identified as potential means of contamination (Trevett *et al.* 2005a; Oswald *et al.* 2007). Additionally, microorganisms have been found to survive and grow in biofilms on the surface of household storage containers, which could possibly influence the microbial quality of water inside the container (Momba & Kaleni 2002; van der Merwe *et al.* 2013).

The present study examines the causal mechanisms of stored water contamination. An observational study was conducted in which a container of drinking water was followed from source to consumption. An enumerator recorded each instance when an object entered, was removed, or added to the water. At each of these events, water samples were collected to determine the loading of microbial contamination. The samples were tested for fecal indicator organisms *Escherichia coli* (EC) and enterococci (ENT), as well as for diarrheagenic *E. coli* (DEC) genes. The study identifies events and practices associated with a decline in water quality after collection from a shared source, and characterizes the variability in water quality throughout the day.

METHODS

Study area and household selection

The study was conducted during March 2009 in the Bagamoyo District of Tanzania, East Africa (6°28′ S 38°55′ E). Ten households, each with at least one child under the age of 5 and a female caretaker, were recruited for the study. These households were in the control group of a larger behavioral intervention randomized-control study conducted in the area. Households were selected by purposive sampling using data from this larger study so as to form a sample with a variety of source water types, stored water quality, and water management practices. The day prior to the observation, when households were being interviewed for the larger study, households were asked if they would be interested in enrolling in the study. Households were told that the study sought to identify ways to improve child well-being and that several drinking water samples would be collected throughout the observation period. If households agreed to participate, a time was set for the enumerators to return to conduct the observation. The research was approved by the Stanford Human Research Protection Program (CA, USA) and the National Institute for Medical Review (NIMR) of Tanzania (Dar es Salaam, TZ). Free and informed consent was obtained for each participating household in the study.

Structured observation

All study observations were conducted by the same two female enumerators, who lived in nearby towns and spoke the same language as the respondents. At one study household, a female Stanford researcher from the USA was

present for half of the observation period. At the beginning of the day (08:00–09:00 h), the enumerator team accompanied an adult person in the household responsible for collecting drinking water to the household's water source. The study participant was observed while she filled a container with water from the source, and the enumerator team accompanied her back to her home. The enumerator team then remained in the home for the course of the day (approximately 8 h), observing interactions with the same container of water collected at the beginning of the observation period. The enumerators recorded all interactions with the drinking water on a handheld personal data assistant (PDA) using a questionnaire developed with The Survey System (TSS) (Creative Research Systems, Petaluma, CA, USA). Specifically, enumerators recorded how and where water collection containers were cleaned, located, and covered. Information on water extraction techniques used by household members was also collected, including whether any hand contact with the water was observed.

Sample collection and processing: water sample collection

Between 6 and 12 water samples were collected from each household's drinking water storage container over the course of the observation period. During initial collection from the source, one water sample was taken from the source directly and then a second was drawn from the container after the respondent filled it. Thereafter, water samples were collected from the container after any interaction with the water. If no interaction occurred for a period of 2 h, a mandatory sample was collected from the storage container.

A sterile pipette was used to extract 350 mL of water from the container and placed in a sterile Whirl-pak sampling bag (NASCO Corp., Fort Atkinson, WI, USA). An enumerator stirred the water with the pipette for 10 s prior to withdrawing the sample. All samples were stored on ice in a cooler and were processed within 6 h of collection.

Sample collection and processing: initial sample processing

In the water microbiology laboratory at the Ifakara Health Institute in Bagamoyo, all of the samples were processed

for the detection and enumeration of fecal indicator bacteria (FIB) using membrane filtration. The samples were processed following USEPA Method 1604 for EC (USEPA 2002a) and USEPA Method 1600 for ENT (USEPA 2002b). Water volumes of 10 or 100 mL were processed using membrane filtration, depending on the turbidity of the sample. Water sample volumes were consistent among samples collected from a single household. The filters from the MI plates used in conjunction with EPA Method 1604 that had EC biomass were removed from the agar, rolled in a 5 mL DNA/RNA free centrifuge tube with 0.5 mL of RNA-later$^®$ (Qiagen, Valencia, CA, USA), and archived at $-20\,^\circ$C until transported to a laboratory at Stanford University at room temperature. Once at Stanford University, the samples were then stored again at $-20\,^\circ$C until molecular processing was conducted (approximately 3–4 months between initial processing and molecular analysis).

Sample collection and processing: chlorine measurements

Chlorine was tested in the field using chlorine strips (Hach Company, Loveland, CO, USA) that allow determination of the presence of free and total chlorine. If a sample tested positive for chlorine, sodium thiosulfate was added to it prior to processing for FIB in order to prevent chlorine-induced cell deactivation.

Sample collection and processing: molecular analyses to detect pathogenic genes of diarrheagenic *E. coli*

Source water samples, samples from the fetching container taken immediately after filling, and water samples from the last extraction event during the observation period were assayed for genes specific to EC pathotypes. The bacterial DNA was extracted from the archived MI filters using a MoBio PowerWater DNA kit (MoBio Laboratories, Carlsbad, CA, USA). Extraction blanks were performed with each set of extractions (9–11 samples per set). The extracted DNA was stored at $-20\,^\circ$C until the multiplex polymerase chain reaction (PCR) assays were conducted. Three multiplex conventional PCR assays were conducted using reagents supplied in the Type-It Mutation Detect PCR Kit (Qiagen). For each 25 µL multiplex PCR reaction, 2 µL of DNA extract

from the samples were used. Each PCR reaction solution included 1× Type-It Multiplex PCR Master Mix (2×), 0.5× Q-solution (95×), 1× Coral Load Dye (10×), and previously published primers for identification of the detection of seven EC pathogenic genes that typically define the following patho-groups: enteroinvasive, enteropathogenic, enteroaggregative, enterotoxigenic, and enterohemorrhagic (Table 1). A no template control and a positive control were run with each multiplex PCR assay. The primer concentrations, annealing temperatures and annealing times are displayed in Table 1 (Pickering *et al.* 2012).

Sample collection and processing: source water control

An acid-sterilized 20 L jug was filled with water at each participant's source, brought to the laboratory, and placed in an area of similar temperature and sunlight intensity as inside the study participant's home. As with the stored water, the water in these containers was sampled every 2 h after being mixed for 10 s using a sterile pipette. This water

served as a control to determine attenuation or re-growth rates of the indicator organisms over the course of the observational period.

Data analysis to determine microbial loadings/removals

Decay rates were calculated for EC and ENT in each household's stored drinking water using the source water controls. The decay rates of each organism is assumed to follow a first-order decay equation:

$$\frac{dC}{dt} = -kC \tag{1}$$

where k is the first order decay rate, C is the FIB concentration, and t is the time. For control data with three or more concentration measurements, a 99% confidence interval was calculated for the decay rate to assess whether the rate was statistically different from zero.

If decay does not influence the mass balance of FIB in the stored drinking water, the loading of FIB to the

Table 1 | Forward (F) and reverse (R) primers and annealing temperatures and times used in multiplex PCR assays to detect diarrheagenic *E. coli* in water samples

	Pathotype of *E. coli*	Gene	Primer name	Primers (5′ to 3′)	Primer conc.[j]	Product size (bp)	Anneal temp.[j]	Anneal time[j]
Multiplex 1	EAEC[a,b,c]	aggR	aggRKs1 F	GTATACACAAAAGAAGGAAGC	0.2 μM	254	60 °C	1.5 min
			aggRkas2 R	ACAGAATCGTCAGCATCAGC	0.2 μM			
	EPEC/EHEC[d]	eaeA	eaeA-F	TCAATGCAGTTCCGTTATCAGTT	0.2 μM	482		
			eaeA-R	GTAAAGTCCGTTACCCCAACCTG	0.2 μM			
Multiplex 2	EHEC[e]	stx1	stx1-F	AAATCGCCATTCGTTGACTACTTCT	0.4 μM	370	58 °C	1.5 min
			stx1-R	TGCCATTCTGGCAACTCGCGATGCA	0.4 μM			
	EHEC[e]	stx2	stx2-F	CAGTCGTCACTCACTGGTTTCATCA	0.2 μM	283		
			stx2-R	GGATATTCTCCCCACTCTGACACC	0.2 μM			
	EIEC[b,f]	ipaH	ipaIII	GTTCCTTGACCGCCTTTCCGATACCGTC	0.5 μM	619		
			ipaIV	GCCGGTCAGCCACCCTCTGAGAGTAC	0.5 μM			
Multiplex 3	ETEC[g,c]	LTI	LTI-F	TCTCTATGTGCATACGGAGC	0.2 μM	322	51 °C	3 min
			LTI-R	CCATACTGATTGCCGCAAT	0.2 μM			
	ETEC[h,i]	STIb	STIb-F	ATTTTTCTTTCTGTATTGTCTT	0.4 μM	192		
			STIb-R	CACCCGGTACAAGCAGGATT	0.4 μM			

[a]Ratchtrachenchai *et al*. (1997).
[b]Toma *et al*. (2003).
[c]Brandal *et al*. (2007).
[d]Vidal *et al*. (2004).
[e]Brian *et al*. (1992).
[f]Sethabutr *et al*. (1993).
[g]Rappelli *et al*. (2001).
[h]Stacy-Phipps *et al*. (1995).
[i]López-Saucedo *et al*. (2003).
[j]Pickering *et al.* (2012).

container due to any interaction with the water (N_{loading}) can be calculated as follows:

$$N_{\text{loading}} = C_2 V_2 + C_e V_e + C_s V_s - C_1 V_1 \qquad (2)$$

where C_e is concentration of water that is extracted if extraction occurred, C_1 is the concentration in the container prior to the interaction, C_2 is the concentration in the container after the interaction, and C_s is the concentration measured in the sample taken after the interaction. V_s is the volume of water taken during sampling and V_1 is the volume of water in the container prior to the interaction. N_{loading} is the loading of FIB in colony forming units (CFU) associated with the interaction. Assuming $C_e = C_2$ and $C_s = C_2$, Equation (4) can be simplified to:

$$N_{\text{loading}} = C_s (V_2 + V_e + V_s) - C_1 V_1 \qquad (3)$$

Given $V_1 = V_2 + V_e + V_s$,

$$N_{\text{loading}} = (C_s - C_1) V_1 \qquad (4)$$

Statistical analysis of FIB loading

Assuming a solution of water is well-mixed, a sample of volume V from the water has a concentration that follows a gamma distribution, $Ga(\alpha, \lambda)$ (Gronewold & Wolpert 2008). The distribution has the shape parameter $\alpha = y + 1/2$, and the rate parameter, $\lambda = V/100$, where y is the CFU observation and V is the volume (units of mL) (Gronewold & Wolpert 2008). A Monte Carlo Simulation was used to determine if the observed loadings, N_{loading}, could be explained by random variability associated with the sampling method. For each simulation, the concentrations (C_s and C_1) were randomly sampled from their corresponding probability distributions (Ga) to calculate N_{loading}. This simulation was repeated 1,000 times to estimate the probability distribution of N_{loading}. In order to determine whether the N_{loading} is statistically different from zero, a two-tailed 99% confidence interval of the simulated N_{loading} was computed.

RESULTS

Observed water interactions

The interactions with stored water documented by enumerators include fetching ($n = 10$), extractions ($n = 64$), and transferring water from one container to another ($n = 1$). None of the households treated their water in any way. The water in the containers that were followed came from public taps connected to municipal piped water networks delivering treated river water ($n = 5$), borewells accessing deep aquifers ($n = 3$), and shallow wells ($n = 2$). Chlorine was detected in water collected from three of the five public taps. On average, the one-way walk time to the water source was 10 minutes (standard deviation = 9.5 minutes). The volume of water fetched was 20 L ($n = 8$) or 10 L ($n = 2$). The water was collected in buckets with lids ($n = 5$), buckets without lids ($n = 3$), and jerricans with a narrow mouth ($n = 2$). Eight of the containers were cleaned by collectors prior to fetching, and each of these containers were cleaned by rinsing with water at the source ($n = 7$) or at home ($n = 1$). Either the female ($n = 9$) or male ($n = 1$) head of household fetched the water. Hands never contacted the water while filling, but leaves were put into the bucket with water by one respondent on the walk home.

In total, 64 extraction events were observed over the course of the study. The water was extracted for drinking ($n = 35$), cooking ($n = 7$), bathing ($n = 3$), dish washing ($n = 7$), and clothes washing ($n = 1$). The female head of household extracted the water for 61% ($n = 39$) of the extraction events, a female child 20% ($n = 13$) of events, a male child 14% ($n = 9$), and a second adult female 5% ($n = 3$). Methods used for extraction included dipping a small cup with a handle (61%, $n = 39$), a small cup without a handle (17%, $n = 11$), a bowl (8%, $n = 5$), a ladle/spoon (5%, $n = 3$), or decanting water from the top of the container (9%, $n = 6$). A hand touched the drinking water eight times out of the 64 extraction events (13%). Each time a bowl was used for extraction, a hand touched the water that had been extracted. The individuals who made hand contact with the drinking water include the female head of household ($n = 6$), a female child ($n = 1$), and a second adult female ($n = 1$).

For seven households, the drinking water container was stored in the home. The other three households stored the water outside the home. For seven households, the container was on the ground; for three households, the container was elevated. One household did not cover their container after any of the extraction events; eight households fully covered their container after all extraction events; and one household either fully or partially covered their container after the extraction events.

Microbial loadings

Initial EC concentrations of the ten control samples were within the assay range of quantification (ROQ) for only four households, and four households had ENT control samples within the ROQ. For the remaining households, FIB concentrations were below the limit of detection ($n = 4$ for EC, $n = 5$ for ENT) and above the limit of detection ($n = 2$ for EC, $n = 1$ for ENT). The control data for nine of the households consisted of three to four concentration measurements at three to four time points approximately 2 h apart. None of the decay rates was statistically different from zero at the 99% confidence interval; therefore, microbial decay within the storage containers was not considered in the loading/removal calculations. For one household, source water collection occurred in the middle of the day, so the control data included only two concentration measurements. A confidence interval around the decay rate could not be calculated, but the rate of growth was slow at $0.02\ h^{-1}$. As serial samplings of the stored drinking water occurred within 2 h or less over the course of a day, the first-order growth would change the FIB

concentration by a small fraction of a CFU. The mass balance of FIB in the drinking water for the household thus did not include the growth rate. It is important to note that the decay rates observed in the controls may differ from decay rates experienced by organisms in the household stored drinking water, as acid-washed, sterile buckets were used for the control experiments. Buckets used by households could contain biofilms that may influence bacterial decay or growth (Momba & Kaleni 2002).

Loadings were calculated for various water interactions. Water interactions are characterized as 'container filling', 'transport', 'extraction', 'transfer', and 'mandatory sampling'. Interactions characterized as 'container filling' refer to the initial filling of the collection container with water from the source. 'Transport' refers to the period between filling the collection container at the source and walking home and placing the container for storage. 'Extraction' refers to interactions in which water was removed from the storage container. 'Transfer' refers to the transferring of water from one container to another. A 'mandatory sampling' is a sampling after 2 h of inactivity with the drinking water.

For each water interaction, there was either a positive FIB loading, a negative FIB loading (i.e. removal), or FIB remained the same. The mean loading of EC and ENT associated with each type of water interaction is provided in Table 2. On average, EC loading was positive when the container was initially filled with source water and during transport of the water from source to the home, but negative during extraction events. ENT loading was on average positive during the filling of the water container and negative during the transport and extraction events. However, there was a large amount of variation as evidenced by the very

Table 2 | Summary of *E. coli* (EC) and enterococci (ENT) loadings and removals by interaction type

Interaction type[a]	N, EC (ENT)	Mean EC loading (SD[b]), units CFU	Mean ENT loading (SD[b]), units CFU	Num. loading events (significant loading)-EC	Num. removal events (significant removal)-EC	Num. loading events (significant loading)-ENT	Num. removal events (significant removal)-ENT
Container filling	9 (9)	9,340 (37,200)	144 (11,300)	5 (1)	2 (1)	4 (0)	3 (1)
Transport	8 (8)	11,400 (33,500)	−6,460 (16,700)	3 (0)	3 (0)	3 (1)	4 (0)
Extraction	55 (55)	−1,340 (16,800)	−257 (16,000)	22 (6)	21 (0)	25 (6)	22 (3)

[a]'Container filling' interactions refer to the filling of the collection container. 'Transport' refers to the time between filling the collection container and walking home and placing the container for storage. The 'extraction' refers to water extraction interactions.
[b]SD refers to standard deviation.

large standard deviation values. Thus, it is more appropriate and informative to evaluate whether various loadings were significantly different from zero using the Monte Carlo simulation.

In general, filling the storage containers did not significantly change FIB concentrations in water; only two of the nine filling events observed resulted in EC changing significantly during filling. In one case a positive, and in another a negative, loading occurred. Similarly, transporting the container from the source to the home did not consistently result in significant loadings. Of the eight observed transporting events, just one resulted in a statistically significant positive loading of ENT to the container. No significant loadings in EC were observed during transport. A number of extraction events resulted in significantly positive loadings of EC and ENT to the water in the storage container. For EC, 6 of 55 extraction events represented significantly positive loadings; there were no significant removals of EC during extractions. For ENT, the results for extraction events were equivocal. Of the statistically significant loading events, more were positive than negative (six compared with three), but both positive and negative loading events were observed. A 'transfer' event occurred only once in the study, and the loading was not statistically significant. Mandatory samplings were taken twice in the study, and there were no statistically significant loadings associated with these samples.

Table 3 presents FIB concentrations in water collected from public taps, borewells, and shallow wells sampled in this study. Geometric means of contamination levels of source water and stored water at the end of the observation period are presented. The significant loading of FIB in Table 3 refers to the cumulative loading of FIB to the stored water over the observation period (i.e. between filling the container at the source to the last observed extraction event). The significant positive loadings for FIB occurred in water collected from improved sources (e.g. public taps and borewells), whereas the significantly negative loadings occurred in water from a shallow well, an unimproved source. For public taps, three of the five water samples had residual chlorine, and the two that did not were the only public tap samples that experienced significant loading of FIB over the observation period.

Diarrheageanic *E. coli* gene analysis

Samples from eight households were analyzed for DEC genes (Table 4). The remaining two households in the study did not have culturable EC present in a sufficient number of samples to be included in any of the pathogenic gene analysis. Samples processed for detection of the DEC genes include 'source water' samples (i.e. water collected directly from the source), 'container filling' samples (i.e. the water from the container immediately after filling at

Table 3 | Comparison of geometric means of *E. coli* (EC) and enterococci (ENT) concentrations (CFU/100 mL) in the stored water and source water across households by source water type

Source water type	Num. of households	Geometric mean of EC(ENT) in source water	Geometric mean of EC(ENT) after all extractions	Chlorine present	Significant loading of EC (ENT) over observation period
Public tap	5	1.99 (2.89)	7.56 (4.38)	3	2 (2)
Borewell	3	101 (123)	193 (113)	0	0 (1)
Shallow well with bucket	2	863 (2,940)	1,010 (2,170)	0	0 (1[a])

[a]Significant removal of enterococci.

Table 4 | Number of samples positive for pathogenic genes of diarrheageanic *E. coli*[a]

%(N)	(+) ipaH (EIEC)	(+) eaeA (EPEC/ EHEC)	(+) aggR (EAEC)	(+) LTI (ETEC)	(+) STIb (ETEC)	(+) stx1 (EHEC)	(+) stx2 (EHEC)
Source water ($n = 5$)	4/5	2/5	1/5	1/5	3/5	2/5	1/5
Container filling ($n = 7$)	7/7	3/7	1/7	3/7	3/7	3/7	1/7
Extraction ($n = 8$)	7/8	3/8	2/8	2/8	4/8	3/8	1/8

[a]Including enteroinvasive (EIEC), enteropathogenic (EPEC), enteroaggregative (EAEC), enterotoxigenic (ETEC), and enterohemorrhagic (EHEC).

the source) and 'extraction' event samples (i.e. the water from the container after the last observed extraction event for the household). Not all sample types for each household were analyzed for DEC genes due to: (1) lack of EC growth on the MI media; and (2) missing data. The DEC data are not available for 'source water' samples from three households and a 'container filling' sample from one household.

Samples for five households were processed for the detection of DEC genes in the source water. For each pathogenic gene tested, at least one source water sample tested positive for the gene. For the *ipa*H and STIb genes, the majority of the source waters tested were positive for the genes (80 and 60%, respectively) (Table 4). Samples from seven households were processed for the detection of the pathogenic genes in the 'container filling' water sample. The samples taken from the storage container immediately after filling showed higher detection of pathogenic genes as compared with the source water samples. There were five instances when pathogenic genes of EC were not detected in the source water but were detected in the 'container filling' sample. This happened twice for LTI, once for *eae*A, once for *stx*1 and once for *stx*2. For one of the instances where initial gene detection occurs in the 'container filling' samples, a significant loading of EC also occurs to the water between the source and immediately filling the container. For the two households that used source water with no EC detected from the membrane filtration procedure, EC was detected in the water after the fetching container was filled and the 'container filling' samples were analyzed for pathogenic EC genes. The gene stx1 is detected in the 'container filling' sample of one household; the gene ipaH is detected in the 'container filling' sample of both households.

'Extraction' samples from eight households were also processed for the DEC pathogenic genes. Pathogenic genes of EC were detected solely in the water after the last observed extraction event (i.e. pathogenic genes not detected in the source water or 'container filling' sample) four times, once for genes aggR, eaeA, stx1, and Lt1. For three of these instances, there were also significant loadings of FIB in the water between the 'container filling' and the last observed extraction event. Inferential statistical tests comparing detection of the DEC genes between the 'source', 'container filling', and 'extraction' samples are not shown due to limited sample size.

For two of the instances when initial EC pathogenic gene detection in the drinking water occurred after collection from the source, the gene sequence was detected in the source water control of the household. Also, there are five instances where EC pathogenic genes were detected at earlier stages in the water sampling pathway, but then the genes were not detected at a subsequent stage.

DISCUSSION

Many different types of household stored water interactions gave rise to contamination. Extracting water from a storage container with a cup (with or without a handle), a ladle, as well as decanting water from the top of the container, were all associated with a significantly positive loading of FIB. Some of these extraction methods (e.g. dipping with a long-handled ladle and decanting the water) have been supported as 'safe' extraction techniques, so the results from this study bring into question the efficacy of these extraction methods to consistently protect stored drinking water quality. The person extracting the water when increases in FIB were observed included both adults and children, suggesting that multiple household members adversely influence stored water quality. Further, the data indicate that hand contact with stored drinking water during extraction was always associated with a positive loading, although no loading was statistically significant ($p > 0.2$).

One significantly positive loading occurred when a storage container was filled by the water source, and concentrations generally increased between the source and the container. Containers were always rinsed with water prior to filling with no hand contact on the inside surface, but participants never cleaned the container with soap. This raises the possibility that dirty containers may be a contamination source, as suggested by other studies (Trevett *et al.* 2005a; Oswald *et al.* 2007). Additionally, for the significantly positive loading associated with the transport of the water home, the container was covered and no objects came into contact with the water during the walk. The positive loading during transport could potentially be explained by biofilms on the surface of the container gradually introducing the contamination to the water on the walk home (Momba & Kaleni 2002; Mellor *et al.* 2012). Behavior change strategies to reduce

drinking water contamination introduced by the storage containers could be explored. For instance, interventions encouraging the proper cleaning of storage containers could be tested for effectiveness at preventing post-supply contamination of drinking water. Alternatively, introducing residual chlorine into the drinking water, whether from the source or at the point-of-use level, could also serve as a mechanism for preventing post-supply microbial contamination in non-turbid waters (Montgomery & Elimelech 2007).

The contamination trends of the two fecal indicator organisms did not always agree. Results for EC and enterococci were in agreement for three significant loading/removal events, which represent only 19% of all significant loading/removal events. Reasons for the inconsistent results for these organisms include differential contamination of EC and ENT on surfaces (i.e. utensils, container) due to their different concentrations in feces (Ervin *et al.* in press) as well as different rates of persistence on surfaces (Pinfold 1990); additionally, different survival rates of the organisms in fresh water environments could be influential (Anderson *et al.* 2005; Walters & Field 2009).

Concentrations of FIB in stored drinking water of households in Bagamoyo, Tanzania, were dynamic and fluctuated over the course of the day as demonstrated by the large standard deviations of the mean FIB loadings (Table 2). Many studies have used one-time measurements of stored drinking water as an outcome indicator of intervention effectiveness, and the results of this study suggest that point FIB concentrations may not serve as a reliable indicator of overall water quality. Some of the variability can be attributed to the detection and enumeration methods of these organisms (i.e. membrane filtration and organism culturing), which, for this study, was accounted for in the Monte Carlo simulation to determine statistically significant loadings (Gronewold & Wolpert 2008). Additionally, human interactions with stored drinking water can have an important influence on the concentrations of FIB in the water, as statistically significant loadings of FIB identified in this study reveal. Also, natural attenuation (Levy *et al.* 2008), regrowth and biofilms (Momba & Kaleni 2002; Mellor *et al.* 2012) could influence the concentrations of FIB found in stored water. Many mechanisms contribute to the variability of stored water quality; therefore, multiple samplings would improve the reliability of water quality measurements. A geometric

mean standard, using a geometric mean of multiple samplings over a specified period of time, would serve as an improved indicator of drinking water quality in a household (Boehm *et al.* 2002; Levy *et al.* 2009).

When considering the statistically significant loadings of FIB that occurred over the entire observation period for each household (i.e. loadings occurring between filling the container and the last observed extraction event), significant positive loadings occurred in waters collected from improved sources (i.e. public taps and borewells), which is consistent with current literature on post-supply contamination (Wright *et al.* 2004). Waters collected from a public tap on the distribution network were consistently contaminated by extraction events (i.e. statistically significant loadings of FIB always occurred) if chlorine was not present, highlighting the benefit of residual disinfectants in water. Those waters with residual chlorine did not become re-contaminated with FIB during the study period, revealing that the use of chemical disinfectants can be effective in sustaining water quality gains of improved, communal water infrastructure through in-home storage of the water.

Assessing the loading of FIB to stored water reveals increases in microbial contamination, but further analysis was conducted to determine if the water interactions introduced genes associated with pathogenic EC to the stored water as well. Pathogenic genes of EC were first detected in the water after filling the collection container as well as after the observed extraction events. The detection of the pathogenic genes in stored drinking water that did not have pathogenic genes when collected at the source reveals that the post-supply contamination of drinking water could potentially represent a health risk. Further work should explore the introduction of pathogenic organisms in stored water to better understand health risks associated with consuming contaminated stored waters. Additional work should also explore if pathogens introduced to the stored water by the household represent a risk to health, as some studies assert that this health risk is minimal (Vanderslice & Briscoe 1993). The detection of pathogenic genes was sometimes inconsistent in the drinking water samples, with a gene no longer being detected in a sample from water that earlier in the sampling pathway tested positive for the gene. These results suggest that concentrations of the EC pathogenic genes could be near the detection

limits of the assays, or that the presence of these genes are dynamic in the stored drinking water.

There are several limitations to the study. When conducting structured observation studies, enumerator presence can influence the behaviors of the study participants (i.e. Hawthorne effect) (Holden 2001). Therefore, the water handling practices observed in the study could have deviated from standard practice; however, there is no evidence to suggest that this occurred. Nonetheless, the main focus of the study is identifying how the observed water handling practices influence microbial contamination in stored drinking water, and we are not attempting to generalize the behavioral practices observed to any population.

Another study concern is the inherent variability in enumerating FIB (Gronewold & Wolpert 2008; Gronewold *et al.* 2013). Although the study attempts to account for the variability using a Monte Carlo simulation, the number of significant loading events could be over-estimated if the variability is larger than what is theoretically expected. In order to better assess the intra-sample variability, future studies should collect replicate grab samples at each time point. Additionally, the current study was small in scale to explore a novel approach to understanding in-home water contamination, but the small sample size introduces limitations to the generalizability and robustness of study conclusions. To further corroborate the study findings, future work would benefit from an increase in sample size. It should be considered that the methods used in this study (i.e. structured observation, serial water sampling) are quite intensive but provide deeper and more refined understanding into how household water management behaviors influence water quality.

CONCLUSIONS

Household interactions with drinking water after collection from a communal water source were found to significantly increase microbial contamination of the water. The data collected are consistent with the idea that storage containers and extraction utensils introduce microbial contamination into stored drinking water. Even decanting the water from the container was found to increase contamination in the water, suggesting that further research is needed to determine whether this method is consistently associated with protection to stored water quality. The role of microbial biofilms on the container inside surface and the microbial environment in the water should also be further explored, as some of the variability of FIB concentrations could be attributed to the behavior of microbial communities in the containers. The current study has several limitations, particularly due to the inherent variability in FIB enumeration and the small sample size, and increasing the sample size in future work would improve the generalizability and robustness of results.

As private water connections for all households is highly unlikely until the distant future, it is important to understand how best to manage the quality of water collected from shared sources. The effectiveness of various container and water extraction methods in reducing FIB contamination of stored drinking water should be explored. Reductions in re-contamination of stored water would, in turn, reduce morbidity and mortality associated with waterborne diseases and allow greater health gains from communal water infrastructure investments.

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