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Coliform Sources and Mechanisms for Regrowth in Household Drinking Water in Limpopo, South Africa

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Abstract

Resource-limited communities throughout the developing world face significant environmental health problems related to the myriad of coliform sources within those communities. This study comprehensively investigated contamination sources and the biological and chemical mechanisms sustaining them in two adjacent communities in rural Limpopo, South Africa. An 8-month study was conducted of household ($n = 14$) and source water quality, measurements of biofilm layers on the inside of household water storage containers and water transfer devices, and also hand-based coliforms and hand-washing effectiveness. A 7-day water container incubation experiment was also performed to determine the biological and chemical changes that occur in a household water storage container independent of human interference. Results indicate that household drinking water frequently becomes contaminated after collection but before consumption (197 versus 1,046 colony-forming units/100 mL; $n = 266$; $p < 0.001$). The most important contamination sources include biofilm layers on the inside of storage containers (1.85 ± 1.59 colony-forming units/cm²; $n = 44$), hands ($5,097 \pm 2,125$ colony-forming units/hand; $n = 48$), and coliform regrowth resulting from high assimilable organic carbon (AOC) levels during storage. A maximum specific growth rate, $\mu_{\max}$, of 0.072 ± 0.003 h⁻¹ was determined for total coliform bacteria on AOC, and a high correlation between AOC concentrations and the growth potential of total coliform bacteria was observed. These results support the implementation of point-of-use water treatment and other interventions aimed at maintaining the safe water chain and preventing biological regrowth.

CE Database subject headings

Drinking water; Water quality; South Africa; Rural areas

Author keywords

Developing countries; Coliform; Water quality; Africa; Bacteria; Public health; Carbon; Organic carbon; Drinking water

Introduction

Worldwide, more than 780 million people lack access to improved sources of drinking water, whereas a further 2.5 billion do not have improved sanitation. Improved water sources include those that are constructed to protect water from outside contamination such as protected springs or boreholes (WHO and UNICEF 2012). Improved sanitation facilities are those that separate human excreta hygienically from human contact and are not shared. In resource-limited settings, these are typically simple pit latrines.

This limited access leads to 1.6 million deaths each year of children under 5 years old (WHO 2006). Repeated episodes of early childhood diarrhea (ECD) lead to serious problems such as stunting (Checkley et al. 2008), long-term cognitive deficits (Niehaus et al. 2002), and lower performance in school (Lorntz et al. 2006). It is therefore imperative that community coliform sources and growth mechanisms be assessed in developing world communities to inform the implementation of interventions aimed at alleviating this burden.

Previous researchers have investigated pathogen sources within developing communities and reported that household drinking water frequently becomes contaminated after collection but before consumption (Wright et al. 2004). In addition, two major meta-analyses found that hygiene, sanitation, and point-of-use water treatment devices were more effective in preventing diarrhea than community water source protection (Fewtrell et al. 2005; Clasen et al. 2007). Others have used complex systems approaches to show that human behavior has a large impact on household water quality (Mellor et al. 2012a).

Corroborating this evidence is the fact that several focused studies have found that point-of-use (e.g., household-level) water treatment technologies are effective at reducing waterborne coliform transmission (Schmidt and Cairncross 2009; Kallman et al. 2011). This is possibly because point-of-use systems treat water that might have been recontaminated during transport or storage. Point-of-use systems are typically simple, low-cost devices that treat water in the home for a typical family either through physical filtration, biological, or chemical means (Schmidt and Cairncross 2009). Ceramic water filters, solar disinfection, and biosand water filters are some of the most common technologies. Alternatively, households can chlorinate their stored drinking water, which can likewise improve drinking water quality (Sobsey et al. 2003).

All of these studies suggest that significant contamination occurs after collection and before consumption but fail to pinpoint or quantify the different coliform sources.

One possible source of contamination are biofilm layers. Biofilm layers form when microorganisms attach themselves to a surface by secreting extracellular polymers. The new structure can create an environmental reservoir for pathogenic microorganisms, making them resistant to antimicrobial treatment (Wingender and Flemming 2011). Some researchers have tried to quantify biofilm layers (Jagals et al. 2003), whereas others have suggested that water may frequently be recontaminated through hand contact (Roberts et al. 2001; Pickering et al. 2010b). However, these studies were conducted under atypical conditions that do not represent average conditions in developing countries. Roberts et al. (2001) had a small sample size of 10 participants and was conducted in a refugee camp with conditions that are atypical. Furthermore, the study by Pickering et al. (2010a) was conducted with a mixed group of teachers, nurses, and mothers in urban schools and health clinics, making those results less applicable to a rural location inside an individual's household. Furthermore, it is possible that hand and water transfer device contamination could contribute to the formation of the biofilm layer on the insides of the water containers, making concurrent measurement highly desirable.

In addition to the known presence of these coliform sources and methods to prevent the consumption of poor quality water, an unanswered question in the literature is the extent to which conditions suitable for coliform growth exist in household water storage containers in the developing world and how that might influence coliform growth or decay.

One possible source for these increased levels is declining residual chlorine concentrations resulting from long storage periods of community tap water that may lead to coliform regrowth if the water has suitable conditions. One study was conducted by LeChevallier et al. (1996) in the United States with a fully modern treatment and distribution system. Systems with low dead-end free chlorine levels were found to have more occurrences of coliform bacteria. Another study by Roberts et al. (2001) found that chlorine levels declined with time in water storage containers, but the experiment was only 6 h long, much shorter than typical storage periods in such settings. Although suggestive, neither of these studies accurately reproduces conditions typical in the developing world.

Coliform growth may also be affected by phosphorus, nitrates, dissolved oxygen (DO), chemical oxygen demand (COD), temperature, or organic carbon (Maier et al. 2009). However, these associations have not been properly assessed in rural, developing world, communities.

Momba and Kaleni (2002) looked at regrowth on galvanized steel and polyethylene coupons suspended in water containers but not at regrowth in the bulk water. A second study by Vital et al. (2010) looked at biological regrowth using precultivated bacteria strains in European water, whose microbiota were likely very different from the microbiota found in a tropical environment. Nonetheless, these studies do indicate that bacterial regrowth might occur in the bulk water low-nutrient environments common in developing world storage containers, and it may depend on the amount of carbon and other conditions.

Therefore, biological regrowth in developing world water storage containers is a potentially important and understudied phenomena in developing world settings. Vital et al. (2010)

assess the extent of possible pathogen growth in controlled environments with three criteria. First, the pathogens must be present in the water. Second, there must be suitable conditions for growth. Lastly, the pathogens in question must compete with the autochthonous flora. A water source's suitability for regrowth can be assessed by measuring its assimilable organic carbon (AOC) concentration (Vital et al. 2010).

AOC is the small (0.1–9%) fraction of total organic carbon (TOC) that can be readily assimilated by bacteria (van der Kooij and Hijnen 1985). It has a strong influence on bacteria regrowth and biofilm growth in previously treated water systems in the developed world (van der Kooij et al. 1992; Escobar et al. 2001; van der Kooij 2002). It can also be used as the limiting substrate concentration when modeling bacteria growth using the Monod equation (Vital 2010):

$$\mu = \frac{\mu_{\max} S}{K_s + S}$$

where S = substrate concentration; K_s = half-saturation constant; and $\mu_{\max}$ = maximum specific growth rate. Although AOC has been studied under controlled environments in developed countries by the aforementioned researchers, no studies have been carried out to assess AOC's presence or impact on biological regrowth in a tropical, developing world setting.

Clearly, there are significant knowledge gaps in scientists' understanding of coliform sources and growth mechanisms in a developing world community. It is also clear that a better understanding of these sources and growth mechanisms can inform the wider development community about ways to reduce coliform transmission in such settings, thereby improving the health of the world's most vulnerable. Therefore, the aim of this study is to investigate coliform sources and regrowth mechanisms in stored household water in a rural, South African community. This study contributes to the literature by investigating multiple different coliform sources in the same communities concurrently. It also investigates the biological and chemical changes that occur within a water storage container over the course of a week to better understand the parameters important for biological regrowth, including levels of AOC.

Methods

Field Site Setting and Household Selection

This study was carried out in the adjacent, rural communities of Tshapasha and Tshibvumo in Limpopo, South Africa. Limpopo Province is South Africa's second poorest and most rural province (90% of its people live in rural areas) (Lehohla 2006, 2011). Diarrhea is the second leading cause of death amongst children younger than 4 years of age (Bradshaw et al. 2005). In addition, diarrhea rates are 1.7 times higher than the national average and have increased 170% between 2003 and 2008 in Vhembe District, Limpopo (Sello 2010).

A prestudy assessment found that community members get water from three primary sources: surface water (SW) from a stream bisecting the two communities, municipal taps

(MT) operated by the local municipality, and a community piped (CP) water system. MT water is treated (including chlorination) by the Mutale municipality and piped to communal standpipes located throughout the communities. The Mutale municipality water supply was recently rated as having excellent drinking water quality compliance in the 2012 Blue Drop study, which assessed drinking water quality throughout South Africa [Department of Water Affairs (DWA) 2012]. However, the MT system is unreliable—for this study, households reported that water does not flow 31.6% of the time ($n = 50$).

CP was a poorly performing development project that was improved through a collaborative effort between University of Virginia and University of Venda students and faculty with substantial community support (Harshfield et al. 2009; Heil et al. 2010). CP consists of a series of pipes that brings stream water down from hills behind the communities to a storage tank, where it may be chlorinated and sent to standpipes in selected households. However, the prestudy assessment found that the water is rarely chlorinated. This system is likewise unreliable with households reporting for this study that they cannot get water 45.4% of the time ($n = 50$).

Forty-nine (or approximately 48%) of the total number of households in Tshapasha and Tshibvumo with children younger than 5 years old were selected to participate in the study. Although logistical and practical constraints prevented random selection, they were chosen to represent as broad a geographic and socioeconomic cross section of the communities as possible and include nearly every household that was willing and able to participate in the study. Among them, a subset of 14 households (9 MT and 5 CP households) was selected to participate in the monthly testing. This smaller subset was chosen for their coparticipation in The Interactions of Malnutrition and Enteric Infections: Consequences for Child Health and Development (Mal-ED) project (Lang 2011) and to represent the MT and CP community water sources. Finally, a second subset of 12 households was chosen from the larger pool that represented the two most common types of water storage containers—narrow and wide neck—and the three water sources. These containers are shown in Fig. 1. These 12 households were asked to relinquish their water storage containers for a 7-day incubation experiment. A Venn diagram describing all three cohorts is given in Fig. 2.

All households were surveyed about their water, sanitation practices, and knowledge in the regional language of Venda. *Good*, *OK*, and *poor* water container washing techniques were assessed by asking participants how they washed their storage containers. Those who reported scrubbing with soap had a good technique. Those who reported either using soap or a scrubbing had an OK technique, and those who just rinsed it had a poor technique. In addition, households were queried about their water treatment practices and latrine prevalence.

Field Sampling Methods

Monthly water sampling in the household as well as measurements of contamination of hands, water containers, and water transfer devices were all measured in the communities.

Monthly water sampling was carried out through household visits. Each household was asked to produce approximately 100 mL of drinking water, which was poured into a

sterilized Whirl-Pak (Nasco) bag containing 10 mg sodium thiosulfate. No accounting was done for potential cross-contamination from household water transfer devices because the stated goal was to measure the quality of water as actually consumed. The term “drinking water” was used because many households also keep presumably inferior quality water for other domestic uses such as washing.

Hand contamination was measured before and after washing by using the protocol described by Pickering et al. (2010a). In brief, the participants were asked to fully immerse one randomly selected hand into a sterilized 2-L Whirl-Pak bag filled with 500 mL of bacteria-free water and agitate it for 30 s. After being asked to wash their hands as they normally would, participants were asked to immerse and agitate their other hand in a second bag for 30 s. There are no strong cultural norms that would make one hand more contaminated than the other in the Limpopo region.

Biofilm buildup was measured using a sterilized swirl protocol (Roberts et al. 2001). Water storage containers were emptied out, and 500 mL sterilized water was introduced. The containers were then capped when possible and vigorously shaken by hand for 15 s. The effluent was then collected and measured for coliform bacteria.

Water transfer devices (typically a cup or ladle) were analyzed by fully immersing them in a 2-L Whirl-Pak bag filled with 500 mL sterilized water and agitating the water for 10 s. The transfer device was then aseptically removed, and the water was measured for coliform bacteria.

All samples were kept on ice in a portable cooler during transportation back to the laboratory. Samples were tested within 24 h.

Water Container Incubation

As previously mentioned, 12 water storage containers were procured from study participants. The 12 containers were divided into three groups of four based on the source water used by the participants (MT, CP, and SW). These containers were then filled with 20 L of water from the participants' primary water source and incubated indoors for 7 d. During this time, the containers were not disturbed except for aseptic sampling. The water maintained a nearly constant temperature of 19°C throughout the experiment.

Analytical Methods

Biological contamination was quantified using m-ColiBlue24 Broth (Millipore, Billerica, MA) as a growth medium to detect total coliform bacteria using membrane filtration. Each sample was first diluted by a 1:10 or 1:20 ratio by using sterilized, unbuffered, deionized water. It was then passed through a 0.45 μ m Fisherbrand (Pittsburgh, PA) water-testing membrane filter, incubated for 24 h at 35°C, and the colonies were counted visually. Periodic blank samples all showed zero colonies.

Coliform concentrations for the hand contamination experiment were normalized and results are reported in terms of colony-forming units/hand. Results for the water storage container biofilm rinse experiment were normalized by container surface area and results are reported

in terms of colony-forming units/cm². Finally, results for the water transfer device were also normalized and reported as colony-forming units/device.

Turbidity was measured using a Hach Turbidimeter with units reported in NTU.

Assimilable Organic Carbon (AOC) was measured with a bioassay and the standard flow-cytometric method (Hammes and Egli 2005). A natural water sample from the river bisecting the communities was collected and filtered using a prerinsed 0.22- μ m syringe filter (BD, Franklin Lakes, NJ) to remove bacteria and interfering particles. The filter was prerinsed with sterilized deionized (DI) water. Filtered water was then reinoculated with 10 μ L/mL of river water and incubated in a rotating incubator at 30°C for 7 days to produce the inoculum.

Experimental samples were collected, of which 15 mL were filtered using prerinsed syringe filters into AOC-free glassware. The sample was then inoculated with 30 μ L of inoculum, after which it was capped and incubated in a rotating incubator at 30°C for 48 h.

Positive controls were prepared similarly using various concentrations of sodium acetate (Fisherbrand, Pittsburgh, PA) in commercially available bottled water. Negative controls contained only bottled water.

All glassware used for collection, processing, and incubation was made AOC free. AOC was removed from glassware by soaking it for 12 h in 0.1 N HCl, rinsing it with deionized water, and heating it to 500°C for 5 h in a furnace. Teflon-coated caps were soaked in a 10% sodium persulfate solution for 1 h, rinsed with deionized water, and air dried.

After incubation, cells were lysed using a Beckman Coulter DNA Prep Lysis Buffer and enumerated using a Beckman Coulter Cytomics FC 500 Flow Cytometer and CXP Software. That unit has a 488 nm, 100 mW argon-ion laser. Green and red fluorescence was collected on the FL1 and FL3 channels, respectively. Instrumental gains were 551 for FL1 and 250 for FL3, and acquisition time was 300 s for each sample.

Total organic carbon (TOC) and free chlorine were measured photometrically using a Hach DR/890 Portable Colorimeter. Hach Method 10129 was used for TOC (Hach 2009). Hach method 10069 which is equivalent to Standard Method 4500-C1-G for drinking water was used to measure free chlorine [Hach 2009; American Public Health Association (APHA) et al. 2012]. Dissolved oxygen (DO) was measured using a Vernier Dissolved Oxygen Probe (DO-BTA), which was interfaced to a computer using a LabQuest Mini. The DO data was collected using *Logger Pro 3* software.

Statistical Analyses

Statistical analyses were carried out using R Statistical Computing Package (www.r-project.org) and Microsoft Excel. Student's *t*-tests, paired *t*-tests, and ANOVA analyses were conducted where appropriate. All statistical tests were done at 95% confidence levels. All boxes in the boxplots represent the median, upper, and lower quartiles. The whiskers represent the lowest and highest data still within a 1.5 $\times$ interquartile range from the median, whereas the outlying circles represent data outside that 1.5 $\times$ interquartile range.

Results

Monthly Household Testing

Water in the communities is obtained from three primary sources: municipal taps operated and chlorinated by the local municipality (MT), a piped water system operated by community members (CP), and surface water (SW) from a nearby river. 57% of participants reported getting their water from the CP system, 25% from MT, and 17% from SW. When a household's primary water source is not working, 63% of the total households use SW, 25% CP, and 12% MT. Water source availability is highly variable, forcing residents to frequently store their drinking water for an average of 3.0 ± 0.6 days ($n = 47$). Although most houses (93.8%) have latrines, few households (18.8%) reported treating their water.

Monthly water testing carried out from December 2011 through July 2012 indicates that a significant increase in coliform levels occur between sources and households. Overall, water quality deteriorated between source and household from 197 to 1,046 colony-forming units/100 mL ($n = 266$; $p < 0.001$) on average for all households surveyed. The MT water system showed an increase from 88 to 1,098 colony-forming units/100 mL ($n = 132$; $p < 0.001$) on average, whereas the CP system also showed a significant increase, 334 to 948 colony-forming units/100 mL ($n = 134$; $p < 0.001$). Data for the MT and CP systems are shown in Figs. 3(a and b), respectively. For comparison, water collected from untreated local surface water sources averaged 938 ± 223 colony-forming units/100 mL ($n = 52$).

It is possible that seasonal variations were observed in the data as can be seen in Fig. 4. Kruskal-Wallis rank sum tests concluded that the monthly variations were significant for the MT system ($\chi^2 = 24.95$; degrees of freedom = 7; p -value = 0.001). The CP variations were likewise significant ($\chi^2 = 16.67$; degrees of freedom = 7; p -value = 0.020). However, these variations could be also produced by sampling error.

Household Coliform Sources

Hands proved to be a significant source of coliforms both before and after hand washing with high concentrations of coliform bacteria being measured in the rinse water. Average before and after levels were 5,097 and 3,903 colony-forming units/hand respectively ($p = 0.320$; $n = 48$). However, dirt was effectively removed as indicated by the significant decrease in turbidity of the rinse water – 2.82 versus 0.81 NTU ($p = 0.014$; $n = 48$). These data are shown in Fig. 5.

Measurements of the biofilm layer on the inside of household water storage containers indicated that a significant and highly variable amount of coliform bacteria are associated with the sidewalls: 1.85 ± 1.59 colony-forming units/cm². This association shows no significant variance with container type: 1.71 and 2.35 colony-forming units/cm², respectively, for narrow neck versus wide neck type containers ($n = 40$; $p = 0.683$). There was no correlation between reported washing techniques and biofilm layers. Those with good, OK, and poor techniques had total coliform levels of 3.02, 0.63, and 0.95 colony-forming units/cm², respectively, $F(2, 36) = 0.966$; $p = 0.390$. Primary drinking water source was also not a significant factor: 1.22 versus 2.49 colony-forming units/cm² for CP versus MT ($t = 2.022$; $n = 40$; $p = 0.528$). Neither reported “time since last washing” nor “collection

frequency” showed a significant correlation to biofilm levels ($R^2 = 0.004$; $p = 0.666$; and $R^2 = 0.009$; $p = 0.556$, respectively). Water transfer devices were also a source of coliforms in many households as indicated by the contamination of the water they were immersed in. The average level of total coliform bacteria found was $2,513 \pm 1,689$ colony-forming units/device.

Water Container Incubation

The incubation experiment of water from different sources stored in containers found a significant increase in total coliform bacteria for the SW and CP containers over the course of 7 days, whereas MT showed little increase. Fig. 6(a) indicates that chlorine levels in MT remained significant throughout the week, whereas those in CP and SW were negligible. Fig. 6(b) shows coliform levels in CP water containers increased from 103 ± 19 to $3,265 \pm 2,495$ colony-forming units/100 mL after the first day. The SW system containers increased from $2,343 \pm 3,746$ to $4,446 \pm 1,958$ colony-forming units/100 mL after the first day and to $8,653 \pm 2,363$ colony-forming units/100 mL after the second day. This corresponds to growth rates of 0.14 h^{-1} for CP and 0.03 h^{-1} for SW. Coliform levels did not rise significantly in either system after the second and third days, respectively, during which time they may have been entering a stationary phase. Water containers used for the MT system saw little coliform growth for the entire 7 days.

The type of storage container made a significant difference with weekly average coliform levels of 1,771 versus 5,072 colony-forming units/100 mL for narrow neck versus wide neck containers (paired t -test; $p = 0.001$) as is shown in Fig. 7. TOC levels decreased throughout the week according to a first-order exponential decay (log-linear fit $R^2 = 0.805$; $p = 0.039$ and $R^2 = 0.750$; $p = 0.026$ for narrow and wide neck, respectively) as seen in Fig. 8(a). Narrow neck containers might possibly have had somewhat lower daily levels than wide ones, but the difference was not significant (2.0 versus 2.3 mg/L for narrow neck versus wide neck paired t -test; $p = 0.156$). DO concentrations decreased according to a first-order exponential decay throughout the week (log-linear fit $R^2 = 0.569$; $p = 0.050$ and $R^2 = 0.623$; $p = 0.035$ for narrow and wide neck, respectively) as is shown in Fig. 8(b). The weekly average DO levels in the narrow neck containers was significantly more than the wide neck type containers (8.2 versus 7.6 mg/L; $p = 0.001$; paired t -test).

Finally, AOC measurements performed on Day 1 indicated levels of 7–840 $\mu\text{g/L}$. These results were poorly correlated with container type. Narrow neck containers had less AOC than wide neck containers (67.3 versus 257.7 $\mu\text{g/L}$ for narrow and wide neck, respectively, $p = 0.409$). There was a significant correlation between the ratio of second to first day coliform levels and AOC concentration ($R^2 = 0.866$; $p = 0.002$) as is highlighted in Fig. 9(a). However, the exclusion of the largest data point reduces the significance of the correlation ($R^2 = 0.550$; $p = 0.091$). The data was fit to a Monod growth resulting in a μ_{max} of $0.072 \pm 0.003 \text{ h}^{-1}$ and K_S of 10.209 ± 2.504 . The best fit is shown in Fig. 9(b).

Discussion

The water sources in these communities represent water sources typically used in many developing world communities (Thompson 2001). However, the surveys indicate that water

collection is complicated by the fact that residents commonly store their water for extended periods. Furthermore, they sometimes switch to alternate, less desirable sources when their primary sources are inoperable for some time. The fact that few residents boil their water coupled with the previously stated data indicates that residents frequently consume contaminated water.

As was found by others (Wright et al. 2004), the increase in total coliform levels recorded between source water and household (irrespective of source water type) indicate that a substantial coliform loading occurs along the water chain between source and consumption. This increase was seen consistently each month. In addition, substantial heterogeneity was apparent even within single households, indicating that households may have good and bad months. Although, since samples were only taken monthly, it is unclear if the measurements represent true seasonal variation or sampling error. These facts indicated that there may be opportunities for improvements and dispersion of best practices within communities, and human behaviors may have a significant effect on drinking water quality (Mellor et al. 2012a).

The biofilm measurements indicated that household storage containers might be a significant and highly variable source of recontamination and are consistent with previous studies by Jagals et al. (2003) conducted in South Africa. Jagals et al. (2003) found median total coliform levels of 158 colony-forming units/100 mL, which is similar to the unnormalized 120 colony-forming units/100 mL found in this study, which used a similar methodology. Even when cleaned frequently, results indicated that the sidewalls contained coliform bacteria. Narrow neck containers, which are more difficult to clean, may have been somewhat cleaner on average in this study since they prevent hand contamination and do not require a transfer device, but the differences are not significant. A similar result was found by others (Jensen et al. 2002; Maraj et al. 2009). Trevett et al. (2005) found a lower percentage, 27% ($n = 8$) versus 63.6% ($n = 44$), of contaminated water transfer devices. This might have been because they were testing for thermotolerant coliforms and had a smaller sample size. The high heterogeneity seen in this study is consistent with previous research and conforms to field observations about the acute differences in observed water container cleanliness.

The results from the hand experiment are likely a result of the inadequate hand hygiene practices of the survey participants. Traditionally, hand washing is done in a communal, open water basin with highly turbid water and without soap. Unprompted to do so, only 12.5% ($n = 48$) of the study participants used soap when washing their hands for this study and 87.5% ($n = 48$) washed their hands in an open basin or bucket. Although this method is adequate for rinsing off dirt, it is ineffective at removing bacteria. Previous studies have shown that improper hand hygiene might therefore be a significant source of coliforms in the community (Pickering et al. 2010b), which might lead to diarrhea (Aiello et al. 2008). The percentage of positive results are higher than has been found previously by Trevett et al. (2005), who found a lower percentage of 44% ($n = 14$) of contaminated hands than was found in this study, 96% ($n = 48$). Improved hand-washing regimes might therefore represent a significant area for coliform reduction in the communities.

Summarizing, water transfer devices and dirty hands likely both contribute to the excess of coliform bacteria found on the sidewalls of wide necked containers, and hence, in the poor household water quality. Therefore, based on the results of this study, narrow neck containers are preferable.

The incubation experiment investigated the growth of coliforms in a household storage container irrespective of human interference. Results indicate that coliform levels increase exponentially during household storage, and this growth might be attributable to AOC concentrations. After this rapid exponential growth, the coliform bacteria appear to enter a stationary phase around Day 3, during which time the coliform concentrations do not rapidly rise. This may correspond to a period in which an essential nutrient is exhausted with the dying cells, providing that nutrient while the cells continue to consume carbon (Maier et al. 2009). TOC and DO levels declined as expected given the marked increase in bacteria levels coupled with a leveling off after Day 3. TOC may have been somewhat higher (although the results were not statistically significant) and DO was lower in wide neck containers, which is consistent with the increased levels of biological growth observed in the wide neck containers. The potential TOC difference could be a result of carbon introduced by water transfer devices or dirty hands. It is not clear from the data what growth stage the coliform bacteria is in. However, the large drop in TOC could be a result of a steady-state situation in which the bacteria are dividing and dying while consuming a considerable amount of carbon. One caveat to these findings are the correlations between wide and narrow neck total coliform and DO levels shown in Figs. 7 and 8(b). It is possible that these correlations could represent a systematic bias. This is particularly apparent in the Day 2 to 3 increase in DO levels in Fig. 8(b). However, this variation is within the 95% confidence intervals and could simply represent random errors.

The long storage periods in this and similar communities throughout the developing world coupled with long collection distances (Mellor et al. 2012b) typical in such settings may mean that coliform regrowth might be a significant and understudied factor in household water contamination. The regrowth seen is consistent with a previous study, in which biological regrowth did occur within 48 h (Momba and Kaleni 2002). Another study by Roberts et al. (2001) showed moderate biological growth for the first 6 h after collection. Neither study measured bacteria levels after these periods. Finally, the lack of any growth in the MT water and the consistently adequate chlorine levels indicate that chlorination is a sufficient method for preventing biological regrowth in such settings. However, the monthly household collections indicate that even when residents get water from previously chlorinated supplies (MT), it can get contaminated in the home likely owing to contaminated hands and water transfer devices.

The maximum specific growth rate found ($0.072 \pm 0.003 \text{ h}^{-1}$) for total coliforms is consistent with the rate previously measured by Vital (2010), who found 0.04 h^{-1} for *E. coli* in natural water at 20°C. The difference could be due to the different bacteria types. In addition, this study looked at indigenous coliforms that must compete with other indigenous bacteria, whereas Vital (2010) looked only at isolated pure culture growth.

In addition, the AOC concentrations measured on Day 1 are strongly correlated with the growth potential as is seen in the strong correlation between AOC concentrations and the ratio of the second to first day coliform levels. Vital et al. (2008) similarly found a positive correlation between AOC and final concentration after a 3-day incubation. Therefore, high AOC concentration might be an important predictor of coliform regrowth potential in the first couple of days. However, the lack of correlation between Day 1 AOC concentrations and the weekly average concentration may suggest that AOC is consumed after the first couple of days when the bacteria concentrations leveled off.

AOC concentrations above 100 $\mu\text{g/mL}$ are significant because they can lead to biological growth of harmful pathogens such as cholera (Vital et al. 2007) or *E. coli* (Vital et al. 2008). 50% ($n = 8$) of the samples had AOC concentrations higher than this value. In addition, Vital et al. (2010) found that the growth potential for a variety of bacterial strains can be 10^6 cells/mL or higher for AOC concentrations in the ranges found in this study. This research is also important because it suggests that higher residual chlorine levels or point-of-use water treatment technologies may be necessary in developing world settings when AOC levels might be elevated (LeChevallier et al. 1996). Because point-of-use drinking water devices treat water in the household ideally just before consumption, they minimize the possibility that water might become recontaminated.

Finally, this study has some limitations that warrant some discussion. First of all, it is unclear how the prevalence and regrowth of the total coliform indicator bacteria corresponds to the prevalence and regrowth of the different diarrhea pathogens. Despite this fact, total coliforms are frequently used by researchers as an indicator of bacterial regrowth (LeChevallier 1990) as well as inadequate water treatment (EPA 1989), which were studied in this paper. Furthermore, the presence of total coliform bacteria can indicate that other harmful bacteria may be present (EPA 1989). Future research should investigate the possibility of pathogen regrowth possibility including viruses, protozoa, parasites, and other known diarrhea-causing pathogens.

Conclusion

Results from this study indicate that water is frequently contaminated after collection but before consumption. Both hands and transfer tools are significant sources of total coliform bacteria and may contribute to the formation of biofilm layers. The biofilm layers and high AOC levels found are likely the source of the coliform regrowth measured. Evidently long storage periods resulting from frequently inoperable water sources coupled with biochemical conditions favorable for regrowth lead to significant recontamination of water sources. The fact that no regrowth was seen in the chlorinated municipal tap water indicates that the residual chlorine provides important protection against recontamination. Narrow neck containers are generally preferable because they reduce the likelihood that container insides might be contaminated by hands or water transfer devices. They also had less biological regrowth in this study. The high heterogeneity in coliform levels seen in this study indicate that best and worst practices are prevalent within communities. The spreading of these best practices might be a means of reducing coliform levels in poorly performing households. In conclusion, interventions that focus on increased reliability of water sources, chlorination,

and point-of-use technologies as well as improved hand-washing techniques and spreading of best practices can all have a significantly positive impact on reducing coliform bacteria within a community.

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Fig. 1.
Household water storage containers most commonly used in the communities: (a) wide neck; (b) narrow neck

Tshapasha and Tshibvumo

~ 410 Households (~102 w/ children under 5)

Fig. 2.

Venn diagram describing the study participants

Fig. 3.

Boxplots of source and household water quality: (a) MT; (b) CP water systems; taps and households are aggregated to the left and right, respectively, of the dashed line in each plot; eight consecutive months of data is given for the participating households

Fig. 4.

Boxplots of eight consecutive months of household water quality in the communities: (a) MT; (b) CP water systems; data indicates significant intermonthly variations ($p = 0.001$ and $p = 0.020$ for MT and CP, respectively), which could possibly indicate seasonal variations

Fig. 5.
(a) Total coliforms; (b) turbidity before and after hand-washing

Fig. 6.

(a) Free chlorine levels for the water containers; MT water maintained significant chlorine levels throughout the week, whereas CP and SW levels remained negligible; (b) total coliform levels throughout the seven day incubation experiment; results indicate significant regrowth in all cases except the MT system

Fig. 7.

Narrow neck containers showed a significantly lower level of total coliform regrowth during the incubation period; data excludes MT containers

Fig. 8.

(a) Wide and narrow neck containers both exhibited a similar decline in TOC concentrations throughout the week, with wide mouth containers having possibly somewhat higher levels—2.0 versus 2.3 for narrow neck versus wide neck, although the difference was not significant; (b) DO decreased uniformly throughout the week, but wide neck containers showed lower daily levels; data excludes MT data

Fig. 9.

(a) AOC concentrations ($\mu\text{g/L}$) versus ratio of second to first day total coliform levels; (b) Monod plot of AOC concentration versus specific growth rate; data excludes MT data