Hands, Water, and Health: Fecal Contamination in Tanzanian Communities with Improved, Non-Networked Water Supplies

AMY J. PICKERING, 1 JENNIFER DAVIS, 1, 8 SARAH P. WALTERS, 1 HELENA M. HORAK, 1 DANIEL P. KEYMER, 1 DOUGLAS MUSHI, 11 RACHELLE STRICKFADEN, 1 JOSHUA S. CHYNOWETH, 1 JESSIE LIU, 1 ANNALISE BLUM, 1 KIRSTEN ROGERS, 1 AND ALEXANDRIA B. BOEHM 1, 11

Emmet Interdisciplinary Program in Environment and Resources, School of Earth Sciences, Stanford University, Stanford, California 94305, Environment and Water Studies, Civil and Environmental Engineering, Stanford University, Stanford, California 94305, Woods Institute for the Environment, Stanford University, Stanford, California 94305, and Sokoine University, P.O. Box 3038, Morogoro, Tanzania

Received November 20, 2009. Revised manuscript received February 15, 2010. Accepted February 27, 2010.

Almost half of the world’s population relies on non-networked water supply services, which necessitates in-home water storage. It has been suggested that dirty hands play a role in microbial contamination of drinking water during collection, transport, and storage. However, little work has been done to evaluate quantitatively the association between hand contamination and stored water quality within households. This study measured levels of E. coli, fecal streptococci, and occurrence of the general Bacteroidales fecal DNA marker in source water, in stored water, and on hands in 334 households among communities in Dar es Salaam, Tanzania, where residents use non-networked water sources. Levels of fecal contamination on hands of mothers and children were positively correlated to fecal contamination in stored drinking water within households. Households characteristics associated with hand contamination included mother’s educational attainment, use of an improved toilet, an infant in the household, and dissatisfaction with the quantity of water available for hygiene. In addition, fecal contamination on hands was associated with the prevalence of gastrointestinal and respiratory symptoms within a household. The results suggest that reducing fecal contamination on hands should be investigated as a strategy for improving stored drinking water quality and health among households using non-networked water supplies.

Introduction

Over 10 million children under the age of five die each year. A large proportion of these deaths are attributed to diarrhea (18%) and the respiratory illness pneumonia (19%) (1). Over 40% of children dying from diarrhea and pneumonia live in sub-Saharan Africa, where lack of improved drinking water sources and poor sanitation are persistent problems (1). Most diarrheal illness is caused by enteric pathogens emanating from feces of an infected individual. The fecal-oral route of pathogen transmission has been well described and comprises several different pathways, including water, food, fingers, soil, and flies (2), yet the relative importance of each pathway is not known.

Several meta-analyses suggest that improved drinking water sources, hand washing, improved sanitation, and household point-of-use water treatment all have the potential to reduce diarrhea in developing countries (3, 4). Of these, hygiene interventions that promote handwashing with soap have shown the highest reductions in diarrhea (45%), nearly twice the reduction recorded from provision of improved water supplies (4). Handwashing has also been shown to be effective at reducing respiratory illness by an average of 21% (5). It should be noted that controversy exists regarding health impact assessments of household water treatment and handwashing interventions because it is often not possible to blind participants, and because behavioral and health outcomes are frequently self-reported (6).

Today over 3 billion people obtain water for their drinking, cooking, bathing, and washing needs from non-networked sources (7). Water is collected in containers at taps located some distance from the home and then transported and stored until consumption. Several studies have shown that household stored water can have higher levels of microbial contamination than source water (8), but causal mechanisms are unclear. It has been suggested that dirty hands play an important role in contaminating water stored in households. Pinfold et al. conducted a study among 10 households in Thailand and found stored water quality was a function of how the stored water was used (i.e., drinking or washing) but not of water quality at the source, suggesting that water handling may be a mechanism of contamination (9). Other studies provide support for this idea with reports of lower levels of contamination in water containers with covers or narrow mouths to prevent hands from entering (8, 10). However, to the best of the authors’ knowledge, a quantitative connection between microbial hand contamination and microbial stored water quality has not been published.

Fecal indicator bacteria (FIB), which occur in high concentrations in feces, are used to indicate the presence of feces in water and on hands and thus signify risk of fecal-oral illness transmission. In developing countries, handwashing with soap has been found to reduce FIB on hands (11–13), and a handful of studies have used FIB on hands as outcome variables to evaluate hand hygiene interventions (14–16). However, little is known regarding the determinants of FIB contamination on hands or how hand contamination relates to other commonly used measures of hand hygiene behavior, stored water quality, and health in developing countries (9, 11, 17, 18).

The present study addresses the connection between hand hygiene, water quality, and health in low-income regions of Dar es Salaam, Tanzania, an area where improved non-networked water supplies have been provided, but anecdotally there have not been large gains in health. The objectives of this study were to identify the characteristics of households...
with high FIB densities on hands, to assess the relationship between FIB on hands and other commonly used measures of hand hygiene behavior, and to investigate the relationship between FIB on hands and FIB in household stored drinking water. In addition, the relationship between the prevalence of diarrheal and respiratory symptoms among households and FIB contamination in stored waters and on hands is explored. Note that this investigation does not consider exposure pathways other than those involving hands and stored water.

Methods
Study Site and Sample Frame. Three hundred thirty-four households in Dar es Salaam, Tanzania (6.2°S, 39.2°E) were enrolled in the study. Households (defined as groups of people that sleep and eat together in a dwelling on a regular basis) with at least one child under five years old were selected using weighted random sampling from three communities in the Ilala and Kinondoni districts. Each household was visited 4 times over three months between July and September 2008. During each visit, data were collected via personal interviews as well as hand rinse and stored water samples. During the same period of time, non-networked water sources in each community were sampled for comparison with the stored water samples. These data were collected as part of an intervention study to test how the provision of water quality and hand contamination results affected behavior, attitudes, knowledge, and quality of stored water and hand rinse samples. The study consisted of a baseline visit, an informational intervention, and two follow up visits. Data from all four visits are used to describe hand contamination and water quality in general, to assess the bivariate relationships between FIB in stored water and FIB in source waters, and between FIB on hands and FIB in household stored water, and to compare concentrations of FIB on hands with other measures of handwashing. We use cross-sectional data from just the baseline visit to document conditions prior to the intervention with respect to determinants of hand contamination and household health.

Household Interview Data. Interviews were conducted with the female head of household to collect data on household water and sanitation services, hand hygiene behavior, and household socioeconomic and demographic characteristics. Prevalence of highly credible gastrointestinal symptoms (HCGS) and significant respiratory symptoms (SRS) were assessed for the 48 h prior to data collection. HCGS and SRS definitions are provided in the SI. Water concentrations are expressed as CFU/100 mL, while EC and FS concentrations are expressed as CFU/2 hands.

Hand Rinse Samples and Hand Cleanliness Data. Enumerators obtained hand rinse samples from the mother (or female caretaker) and up to three children under five during each household visit. Before obtaining a hand rinse sample, enumerators asked mothers how much time had elapsed since she and each participating child had last washed their hands. The presence of visible dirt on the subjects’ palm, fingertips, and underneath the fingernails was recorded as “very dirty,” “somewhat dirty,” or “no visible dirt.” Hand rinse samples were obtained by having the subject insert hands, one at a time, into a 69 oz. Whirl-pak bag (NASCO Corp., Fort Atkinson, WI) containing 350 mL of sterile water. Details of the method can be found elsewhere (12). Hand rinse samples were placed in a cooler on ice and transported to the laboratory for microbial analyses.

Stored Water Samples and Water Management Practices. Between 1 and 2 samples of stored water used for drinking and cooking were collected during each household visit. Enumerators inquired whether and how the water had been treated and how long the water had been stored in the container. Respondents were asked to identify the community water source from which the stored water had been collected. Enumerators documented whether or not the stored water container was covered and asked each respondent to extract water the way they usually would and pour it into a sterile 27 oz. Whirl-pak bag. Sodium thiosulfate was added to the water sample bag immediately prior to collection as a precaution to neutralize any chlorine present in the water. Samples were stored in a cooler on ice and transported to the laboratory for microbiological analyses.

Community Water Sources. Between 29 and 38 unique drinking water sources were sampled in each of the three communities. Community drinking water sources included public and private borewells that sold water, private municipal water taps that sold water, and water sold by street vendors using carts or tanker trucks (hereafter referred to as ‘vended water’). Samples were collected using aseptic technique using a sterile 27 oz. Whirl-pak bag. Sodium thiosulfate was added to the sample bag immediately before collection for all samples obtained from municipal or vended water as well as to other sources the enumerators had reason to believe contained chlorine. Samples were stored on ice and transported to the laboratory for microbial analysis.

Enumeration of *E. coli* (EC) and Fecal Streptococci (FS). All hand rinse and water samples were processed using membrane filtration within four hours of sample collection using Standard and EPA methods (Table 1). FS were enumerated using standard method 9230C (19). EC were enumerated using MI media (BD Difco, Franklin Lakes, NJ) following EPA Method 1604 (20). Further details are in the SI. Water concentrations are expressed as CFU/100 mL, while hand rinse concentrations are expressed as CFU/2 hands. Discrepancies between samples run for EC and FS and the total collected are due to lost or spilled samples.

<table>
<thead>
<tr>
<th>Total Samples Collected and Run for <em>E. coli</em> (EC), Fecal Streptococci (FS), and the Bacteroidales Marker (BAC)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
</tr>
<tr>
<td>153</td>
</tr>
</tbody>
</table>

*Discrepancies between samples run for EC and FS and the total collected are due to lost or spilled samples.

Results
Household and Respondent Characteristics. The average household size was 5.3 people, with 56% living in multifamily houses. Twenty-five percent (25%) reported more than one child under five, and 43% reported at least one infant (defined as 1 year or younger) in the household. The median amount of money spent on household regular expenses (e.g., food, transport) per week was 5833 Tanzanian Shillings (TSH) ($4.20) per person. Female respondents in 23% of households had completed more than primary school education. Only 8% of households had a private improved water source, such
as a borewell or municipal water connection. Other water sources accessed by households included a neighbor’s municipal water tap (21%), neighbor’s borewell (54%), public municipal water tap (5%), public borewell (29%), and vendors (14%); 39% of households reported using more than one water source. The median quantity of water collected per person per day was 48 L. Twenty-two percent (22%) of mothers expressed dissatisfaction with the quantity of water available for household hygiene purposes. All households reported using a private or shared sanitation facility, of which 52% were equipped with a septic tank or improved pit with lining (hereafter collectively referred to as “improved toilet”). At baseline, 3% of households reported that at least one household member with HCGS within the last 2 days. The prevalence of SRS was higher with 17% of households reporting at least one household member with symptoms.

**Contamination levels: Hands.** The mean \( \log_{10} \) concentrations of EC and FS found on hands were 3.1 (SD 1.0, \( N = 2027 \)) and 4.5 (SD 0.8, \( N = 2032 \)) log\(_{10}\) CFU/2 hands, respectively. The *Bacteroidales* marker was found in 50% of the 50 hand samples tested (Figure 1). There was no significant difference in bacterial concentrations found on hands of household members. Percentage of samples tested that were positive for the general *Bacteroidales* marker is represented by the proportion of the circle shaded black for each type of sample (source water, stored water, and hands). Error bars display standard error of the \( \log_{10} \)means.

**Determinants of Hand Fecal Contamination.** Multivariate linear regression was used to assess the association between the \( \log_{10} \) mean FIB of all hand rinse samples obtained from a household at baseline and household characteristics (Table 2). The \( \log_{10} \)-mean of all hand rinse samples from the three most common water sources was 0.2 and 0.6 \( \log_{10} \)CFU/100 mL, respectively, with 31% of EC samples and 13% of FS samples below the detection limit. The \( \log_{10} \)-mean of EC and FS in vended water sources was 0.1 and 0.6 \( \log_{10} \)CFU/100 mL, respectively, with 88% of EC samples and 40% of FS samples below the detection limit. There was a significant difference in FS and EC concentrations between source types (ANOVA, \( P < 0.05 \)), but the only significant pairwise comparison was between FS in borewell water and municipal tap water with borewells containing lower FS (Table S2, Tamhane’s T2 post hoc, \( P < 0.05 \)). The general *Bacteroidales* marker was found in 28% of the borewell samples tested (Figure 1, Table S2).

**Hand Contamination and Other Measures of Hand Hygiene.** Levels of FIB on hands were compared to other commonly used measures of hand hygiene behavior, including self-reported handwashing behavior, presence of soap in the home, and presence of visible dirt on hands. No association was found between self-reported behaviors and mean levels of FIB on hands in households (baseline data only). Also, no difference was found in hand contamination levels between those households with observed soap at baseline (73%) and those without. Hands reportedly washed within the past hour had an average of 0.1 \( \log_{10} \)CFU/2 hands less EC (\( t = -2.78, df = 2025, P = 0.005 \)) and 0.1 \( \log_{10} \)CFU/2 hands less FS (\( t = -2.54, df = 2030, P = 0.011 \)) compared to those washed more than one hour ago (hand rinse samples from all household visits included; see the SI for results for adults versus children).

Visible dirt observed on the subject’s palm, fingerpads, or underneath their nails was significantly related to both EC and FS on hands (Figure S1, Table S3). Hands with palms, fingerpads, or underneath the fingernails classified as “very

![FIGURE 1. Log10-mean concentrations of E. coli (EC) and fecal streptococci (FS) in source waters, household stored waters, and on hands of household members. Percentage of samples tested that were positive for the general *Bacteroidales* marker is represented by the proportion of the circle shaded black for each type of sample (source water, stored water, and hands). Error bars display standard error of the log10-means.](image-url)
dirty” had higher FIB levels than “somewhat dirty” hands, and “somewhat dirty” hands had higher FIB levels than hands with “no visible dirt” (ANOVA, $P < 0.001$, all Tamhane’s T2 post hoc multiple comparisons $P < 0.05$). Typically, hands with visibly clean palms had 60% less CFU EC and 40% less CFU FS per two hands than hands with visibly dirty palms. For children, the magnitudes of the difference in concentrations of EC and FS between hands with visible dirt (present on any part of the hand) and hands with no visible dirt were greater than this same comparison made among mother’s hands (Table S4).

Hand Contamination Levels and Stored Water Quality. The water quality of household stored water samples was compared to the mean water quality of the source from which they were collected. Household stored water was significantly more contaminated than water collected directly from the sources, when analyzed by water source type (Figure 1, independent sample $t$ tests, $P < 0.05$, Table S5). The water quality of 191 stored water samples was compared to the water quality of the exact source from which they were collected. For the comparison, the sources were characterized by their log$_{10}$-mean EC and FS concentrations as determined by approximately 4 samples collected at fixed intervals throughout the study. On average, household stored water had 1.4 log$_{10}$ CFU more EC per 100 mL (df = 190, $t = 19.26$, $P < 0.001$) and 1.8 log$_{10}$ CFU more FS per 100 mL (df = 189, $t = 24.83$, $P < 0.001$) than the source from which it was collected. There was also a significant difference between occurrence of the Bacteroidales marker in stored waters and source waters with stored samples having approximately twice the likelihood of marker occurrence ($\chi^2 = 4.92$, $P < 0.05$, df = 2, Figure 1).

The quality of the stored water was compared between households that used different water management techniques. The presence of a cover was not associated with bacterial levels in the stored waters (99% of sampled stored waters had a cover, independent samples $t$ test, $P > 0.05$). Forty percent (40%) ($N = 242$) of sampled stored waters were reported by respondents to have been treated in some way, and 84% ($N = 355$) of these samples were reported to have been treated by boiling. There were no significant differences in concentrations of EC or FS between samples that had been reported boiled or treated in any way and those reported untreated (independent samples $t$ test, $P > 0.05$). The most common method of extraction observed during sampling (94%) was dipping a nearby cup or mug by hand into the water container. Stored water extracted by hand dipping had 0.3 log$_{10}$ CFU/100 mL more EC ($P < 0.05$) and 0.4 log$_{10}$ CFU/100 mL more FS ($P < 0.05$) than stored water extracted by pouring or release through a spigot.

During each household-visit, the quality of a household’s stored water was compared with the mean contamination level of the household’s hand rinse samples. There was a significant correlation between the log$_{10}$-mean FIB in house-
TABLE 3. Binary Logistic Regression Models Assessing the Relationship between Health and Fecal Indicator Bacteria (FIB) on Hands and in Household Stored Drinking Water*

<table>
<thead>
<tr>
<th>variable</th>
<th>HCGS, n = 277, Cox &amp; Snell pseudo $R^2 = 0.05$</th>
<th>SRS, n = 277, Cox &amp; Snell pseudo $R^2 = 0.04$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>SE*</td>
</tr>
<tr>
<td>constant</td>
<td>-17.0</td>
<td>7.11</td>
</tr>
<tr>
<td>mother has more than primary education*</td>
<td>0.68</td>
<td>0.95</td>
</tr>
<tr>
<td>improved toilet*</td>
<td>-1.00</td>
<td>0.80</td>
</tr>
<tr>
<td>single home*</td>
<td>-1.56</td>
<td>0.64</td>
</tr>
<tr>
<td>regular weekly expenses*</td>
<td>0.13</td>
<td>0.51</td>
</tr>
<tr>
<td>EC in stored drinking water*</td>
<td>0.36</td>
<td>0.43</td>
</tr>
<tr>
<td>FS in stored drinking water*</td>
<td>-0.40</td>
<td>0.19</td>
</tr>
<tr>
<td>EC on hands of household members*</td>
<td>-0.10</td>
<td>0.20</td>
</tr>
<tr>
<td>FS on hands of household members*</td>
<td>0.28</td>
<td>0.09</td>
</tr>
</tbody>
</table>

* Binary variable with values of 0 and 1. a In of TSH per person. c log10 CFU/100 mL. d Mean log10 CFU/2 hands.

Three indicator organisms were used in this study: E. coli, fecal streptococci, and the Bacteroidales marker. The high occurrence and concentrations of all three indicators support an assertion that observed contamination was fecal. However, it cannot be ruled out that soil contributed a portion of the observed bacteria to waters and hands (25). Future work that documents the presence of pathogens on hands and stored waters, rather than indicator organisms, would be useful for confirming sources of contamination and health risks.

Household characteristics associated with fecal contamination on hands included whether the mother had completed primary education, use of an improved toilet, having an infant in the household, and a mother’s dissatisfaction with the quantity of water available for hygiene purposes. Interestingly, household expenditure per person was not found to be associated with FIB levels on hands of mothers and children. A mother’s dissatisfaction with the amount of water available for hygiene purposes was associated with higher FS levels on her own hands as well as her children’s hands. This finding suggests that water quantity may be an important barrier to handwashing and bathing for these households. Overall, the multivariate models explained a relatively low percent of the variation in FS and EC on hands (12% and 4%, respectively).

Self-reported handwashing behavior was poorly correlated to FIB levels on hands. One explanation for this result is that self-reported handwashing data are unreliable, as suggested by others (26, 27). However, visible dirt on hands was highly correlated to FIB levels, supporting further investigation of its use as a possible indicator of hand hygiene. Future work should evaluate the correlation between visible dirt and observed handwashing behavior (e.g., by structured observation) as well as the correlation between observed handwashing behavior and levels of FIB on hands.

A notable finding from this study is that levels of FS on hands were significantly associated with prevalence of gastrointestinal and respiratory symptoms, while FS contamination in stored water was not. For every 1-log10 increase in mean levels of FS on hands of household members, a household was 10 times more likely to report having a household member with HCGS. The same change in FS was also associated with a household being twice as likely to report SRS. A discussion addressing the limitations of the health data used in this study can be found in the SI. The finding that households with lower levels of EC in hand rinse samples were more likely to report an SRS (the opposite association of what was found for FS) suggests that further research is needed to determine what factors or behaviors may differentially affect these indicators. It should be noted that these correlations do not demonstrate causal relationship. Additional work must be undertaken to confirm the observed associations and elucidate underlying causal mechanisms. However, the results suggest that hands may serve as an important intermediary for the transmission of fecal-oral pathogens.

Supporting Information Available
Definitions of symptoms, additional details on the microbial, molecular, and statistical methods employed, hand contamination results by gender and age, limitations of using self-reported health data, Tables S1–S6, and Figure S1. This material is available free of charge via the Internet at http://pubs.acs.org.

Acknowledgments
This work was supported by funding from Stanford University through Woods Institute for the Environment, the Presidential Fund for Innovation in International Studies, Vice Provost for Undergraduate Education, and the School of Earth Sciences. We thank Fred George Njega, staff of the Health & Environmental Rescue Organization, members of our lab and field teams, and participating households for assistance with this research.

Literature Cited


(20) USEPA. Method 1604: total coliforms and escherichia coli in water by membrane filtration using a simultaneous detection technique (MI medium). 2002b (EPA-821-R-02-024).


ES903524M