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Bacterial hand contamination among Tanzanian mothers varies temporally and following household activities

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Summary

OBJECTIVE To characterize mechanisms of hand contamination with faecal indicator bacteria and to assess the presence of selected pathogens on mothers' hands in Tanzania.
METHODS A household observational study combined with repeated microbiological hand rinse sampling was conducted among 119 mothers in Dar es Salaam, Tanzania. All hand rinse samples were analysed for enterococci and *Escherichia coli*, and selected samples were analysed for genetic markers of *Bacteroidales*, enterovirus and pathogenic *E. coli.*
RESULTS Using the toilet, cleaning up a child's faeces, sweeping, cleaning dishes, preparing food and bathing were all found to increase faecal indicator bacterial levels on hands. Geometric mean increases in colony forming units per two hands ranged from 50 (cleaning dishes) to 6310 (food preparation). Multivariate modelling of hand faecal indicator bacteria as a function of activities recently performed shows that food handling, exiting the household premises and longer time since last handwashing with soap are positively associated with bacterial levels on hands, while bathing is negatively associated. Genetic markers of *Bacteroidales*, enterovirus and pathogenic *E. coli* were each detected on a subset of mothers' hands.
CONCLUSIONS *Escherichia coli* and enterococci on hands can be significantly increased by various household activities, including those involving the use of soap and water. Thus, faecal indicator bacteria should be considered highly variable when used as indicators of handwashing behaviour. This work corroborates hands as important vectors of disease among Tanzanian mothers and highlights the difficulty of good personal hygiene in an environment characterized by the lack of networked sanitation and water supply services.

keywords hand hygiene behavior, handwashing, faecal indicator bacteria, Tanzania, pathogens

Introduction

Every day more than 22 000 children die before they reach the age of five (UNICEF 2010). Diarrhoea and the respiratory infection pneumonia are the two main causes of global child mortality and together account for one-third of under-five child deaths (UNICEF 2010). Hands are an important infection transmission pathway, and interventions promoting hand hygiene reduce gastrointestinal and respiratory illnesses by an average of 31% and 21%, respectively (Aiello *et al.* 2008). It follows that widespread interest exists among international health and development organizations in designing and implementing hand hygiene promotion programmes. Such programmes present valuable opportunities for learning what intervention features, characteristics and messages are most likely to result in hand hygiene behaviour change. Unfortunately, rigorous evaluation of such programmes is impeded by the lack of valid and affordable indicators that can be used to measure adherence to hand hygiene behaviours in the field.

Microbiological hand sampling, specifically the quantitative measurement of faecal indicator bacteria on hands, has been used to measure the effectiveness of hand hygiene promotion programmes in developing countries (Pinfold 1990b; Luby *et al.* 2001). A few studies have found an association between measured faecal indicator bacteria on hands and diarrhoeal illness, but a causal relationship has not been established (Luby *et al.* 2007; Pickering *et al.* 2010b). Little is known about the causal mechanisms of hand contamination with faecal indicator bacteria in developing countries or how concentrations on hands change over time, which renders faecal indicator bacterial levels on hands difficult to interpret as indicators of either hand hygiene behaviour or health risk.

Escherichia coli and enterococci are indicator bacteria commonly used to assess faecal contamination of hands. The presence of *E. coli* and enterococci in soil, however, makes it difficult to draw definitive conclusions about faecal contamination (Hardina & Fujioka 1991). *Bacteroidales* are anaerobic faecal bacteria that are increasingly being used to assess faecal contamination because their faecal specificity is believed to be greater than *E. coli* and enterococci. Only one study has reported the occurrence of the general *Bacteroidales* marker on hands of Africans (Pickering *et al.* 2010b), and the human-specific marker has not yet been tested in hand rinse samples. Furthermore, no published work has reported measuring pathogens on hands in Africa. The detection of specific pathogens and *Bacteroidales* concurrently with faecal bacteria on hands would provide valuable insight into the appropriateness of faecal bacteria as indicators of disease risk in Africa.

Two of the most common methods to measure handwashing behaviour are in-person interviews and structured observation. Evidence has been accumulating that self-reported data are unreliable and subject to social desirability bias (Curtis *et al.* 1993; Manun'ebo *et al.* 1997; Biran *et al.* 2008). Structured observation is considered to be the most reliable method, but it can be time consuming, expensive to do on a large scale, and participants have been found to alter their normal behaviour as a result of being observed (Cousens *et al.* 1996). Development of more reliable, objective methods to measure hand hygiene behaviour is needed, particularly indicators that can be obtained by rapid observation or through microbiological hand sampling.

This study explores how faecal bacterial levels on the hands of Tanzanian mothers with young children change during a typical day's events. Specifically, the effect of particular activities on mothers' hand faecal bacterial levels is evaluated through observation and repeated hand rinse sampling. The work also examines the association between faecal indicator bacteria on hands and two low-cost indicators that have not been previously used to measure hand hygiene: turbidity and pH of hand rinse samples. A final objective is to assess the presence of specific pathogens known to cause diarrhoea on mothers' hands and determine whether their presence is associated with the levels of faecal indicator bacteria or *Bacteroidales* markers.

Methods

The study was conducted in a low-income urban community within Dar es Salaam, Tanzania, during July 2009. Households in the study community with at least one child under the age of five and a female caretaker were randomly selected and recruited by enumerators to participate. In total, 119 households were enrolled and assigned to one of two cohorts. Informed consent was obtained from each female caretaker of the child under 5 in the household prior to enrolment. The study was approved by the Stanford Human Research Protection Program and Muhimbili University's Institutional Review Board.

Cohorts

One cohort of 22 mothers participated in an 8-h structured observation while they performed normal daily activities (observation cohort). During the structured observation period, two enumerators each took 1 4-h shift to observe and document each activity the mother performed, including the time of day, duration in minutes and materials the mother touched. If the mother washed her hands, the enumerator documented the water source, whether soap was used, and how the hands were dried. To assess hand contamination over time, hand rinse samples were collected from the mother at 2-h intervals, with the first obtained at the beginning of the structured observation period. In total, five hand rinse samples were collected per mother in the observation cohort.

The second cohort of 97 mothers was enrolled in a shorter observational study that evaluated a specific activity (activity cohort). The caretaker was asked to wash her hands with soap and water in the way that she normally would, and then she was requested to carry out one of the following activities: sweeping, food preparation, dish washing, bathing, cleaning the toilet, defecation, urination or cleaning up after a child who had defecated. The enumerators collected a total of three hand rinse samples from activity cohort subjects: prior to handwashing, immediately after handwashing and immediately after the activity.

A 'sitting' group of 10 mothers was also enrolled as a control, in which each mother held her hands still on a clean paper towel for 10–15 min.

Microbial sampling

Samples were obtained from mothers using the hand rinse sampling method (Pickering *et al.* 2010a), during which both hands were placed consecutively in one bag. Each hand sample bag contained 350 ml of Uhai™ bottled drinking water pre-screened for the absence of target faecal indicator bacteria and dosed with sodium thiosulfate. Hand rinse samples were placed in a cooler on ice and transported to the laboratory for analysis within 4 h of sample collection. All samples were processed via membrane filtration to enumerate enterococci using mEI media per EPA Method 1600 (USEPA 2006) and *E. coli* using MI media per EPA Method 1604 (USEPA 2002). See the online Supporting Information for details on volumes filtered and detection limits.

Calculation of ΔFIB

The hand rinse sampling method used in this study removes a fraction of faecal indicator bacteria (FIB) from hands. To characterize this fraction (f), we collected two sequential hand rinse samples from a cohort of 16 mothers recruited from a different community within Dar es Salaam. The fraction of bacteria removed during the first rinse was calculated from the following expression:

$$f = 1 - \frac{M_2}{M_1} \quad (1)$$

where M_1 refers to the number of faecal indicator bacteria eluted by the first hand rinse and M_2 refers to the number of bacteria measured by the second hand rinse sample, see Supporting Information for details on derivation of f. This expression for f assumes that the same fraction of bacteria is eluted in each rinse; an assumption revisited in the discussion.

To determine the change in faecal indicator bacteria on a mother's hands during specific activities (ΔFIB), the following equation was developed:

$$\Delta[\mathrm{FIB}] = [\mathrm{FIB}]_{\mathrm{Before}} + \frac{[\mathrm{FIB}]_{\mathrm{After}}}{f} - \frac{[\mathrm{FIB}]_{\mathrm{Before}}}{f} \quad (2)$$

where $[\mathrm{FIB}]_{\mathrm{Before}}$ refers to the number of bacteria measured via the hand rinse sample method before the activity or time period of interest, $[\mathrm{FIB}]_{\mathrm{After}}$ refers to the number of bacteria measured by the sampling method after the activity, $\Delta[\mathrm{FIB}]$ refers to the actual change in bacteria during the activity or time period of interest, and f is defined previously.

Molecular analysis

A subset (N = 78) of baseline (first) hand rinse samples obtained from each participant were screened for the general and human-specific DNA markers in *Bacteroidales* as well as for an RNA marker from human enterovirus. For each sample, 70 ml was filtered through a 47-mm, 0.45-μm Millipore HA filter (Fisher Scientific, Pittsburg, PA, USA) (Fuhrman *et al.* 2005). Filters were placed in 2 oz Whirl-pak (NASCO Corp., Fort Atkinson, WI, USA) bags, sealed and stored at −20 °C for the duration of the field project. Samples were transported back to Stanford, California, USA, at room temperature and placed immediately at −80 °C upon arrival. DNA and RNA were extracted from filters. See the Supporting Information for details of nucleic acid extraction methods, PCR and RT-PCRs and thermocycler parameters used.

In addition, 30 hand rinse samples were screened for the virulence genes associated with enterotoxigenic (ETEC), enteroaggregative (EAEC) and enteropathogenic (EPEC) *E. coli* using molecular methods. Genes encoding the toxins *elt* and *Stb1* of ETEC, *eae* of EPEC and *aggR* of EAEC were targeted (Table S1). Samples chosen for *E. coli* screening included 20 samples (10 pairs) obtained before and after observed handwashing with soap as well as 10 samples obtained from two households over a full 8-h observation at a household (two sets of five). Membrane filters containing *E. coli* biomass grown from hand rinse samples were removed from MI agar and placed in Whirl-pak bags at −20 °C for the duration of the field project and transported to Stanford as described for HA filters. DNA was extracted from filters using the Mo Bio PowerMax Soil DNA Isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) following modified manufacturer's directions (see Supporting Information). Details of the PCRs and thermocycler conditions are included in the Supporting Information.

Data analysis

All hand rinse sample results are reported per two hands. Faecal indicator bacterial concentrations were log base 10 transformed for all analysis, except inputs for the calculation of ΔFIB (Equation 2). The Pearson correlation coefficient (r_p) was used to describe the linear correlation between *E. coli*, enterococci, turbidity and pH. Independent sample t-tests were used to assess the relationship of faecal indicator bacterial levels on hands between hands positive for a molecular marker versus those that were negative. The chi-squared test was used to assess the association between the presence of enterovirus and *Bacteroidales* markers on hands.

To investigate how activities and hand hygiene affect the levels of faecal indicator bacteria on hands over time, the structured observation data from each mother in the observation cohort were aggregated by 2-h periods and matched with the hand sample obtained at the conclusion of each 2-h period. The resulting data set included four hand rinse samples from each household matched to the observation data from the preceding 2 h. Faecal indicator bacterial levels on hands were then modelled as a function of activities performed during the 2-h period, using binary independent variables to indicate whether a specific activity had been performed. Generalized estimating equations were used to account for clustering at the subject level (Hanley *et al.* 2003). Statistical analysis was performed with PASW Statistics (SPSS Inc., Chicago, IL, USA), and P-values <0.05 are considered significant for all analyses.

Results

The 119 participating respondents had a mean age of 29. The average household size was 5, and it was common for several families to be living within one housing unit. Only 10% of households had a water source located on their premises; the median time spent collecting water each day was 34 min. Almost all (98%) households have access to a private latrine, but most (88%) share this facility with an average of 4 other families (Table S2).

At the start of each observation period, enumerators documented the presence of visible dirt underneath the mothers' fingernails at 68% of households, and on the palm of the mother's hand at 50% of households. Enumerators also reported the fraction of respondents that could show them a specific place at their home designated for handwashing after using the toilet (60%) and before preparing food (37%). Enumerators observed the presence of soap at 50% of these handwashing stations; water was readily available at 43% of the locations. See Supporting Information for additional data and analysis of hand hygiene behavior indicators.

Faecal bacteria, pH and turbidity

Mothers were found to have a mean of 3.50 log CFU *E. coli* per two hands (SD 0.72, $N = 117$) and mean of 3.03 log CFU enterococci per two hands (SD 0.81, $N = 118$) at the start of structured observations. Hand rinse samples had a mean pH of 7.7 (SD 0.2, $N = 119$) and a mean turbidity of 1.34 log NTU (SD 0.3, $N = 119$). Turbidity was positively correlated with enterococci ($r_p = 0.25$, $N = 396$, $P < 0.001$) and *E. coli* ($r_p = 0.26$, $N = 398$, $P < 0.001$), while pH was negatively correlated with enterococci ($r_p = -0.23$, $N = 396$, $P < 0.001$) and *E. coli* ($r_p = -0.12$, $N = 398$, $P = 0.015$).

Effect of activities on bacteria

Table 1 presents the results of enterococci in hand rinse samples from the observation cohort, modelled as a function of daily activities and time elapsed since last handwashing with soap. Food preparation, exiting the household premises and increased time since last handwashing with soap were found to increase enterococci levels on hands, while bathing decreased levels of enterococci. Washing laundry was weakly associated with lower enterococci on hands, whereas using the toilet/cleaning up a child's faeces, dishes and sweeping did not significantly affect enterococci. The same model with *E. coli* as the dependent variable is not significant (Table S3). Activity frequencies and materials touched by observation cohort mothers are reported in the Supporting Information.

Based on repeated hand rinse sampling, the median value of f_{ec} was 0.52, and the median value of f_{ent} was 0.44. These median values were used in Equation 2 to calculate ΔFIB

Table 1 Generalized estimating equations of log enterococci on hands, modelled as a function of observed activities (binary variables) and time since last handwashing with soap during a 2-h period preceding hand rinse sampling. Model coefficients (β), standard errors (SE) and *P*-values (*P*) are shown; model quasi-likelihood under independence criterion (QIC) = 64

Variable	β	SE	*P*
Constant	2.59	0.38	<0.001**
Going outside	0.51	0.13	<0.001**
Food preparation/eating	0.84	0.32	0.008**
Use toilet/clean up child faeces	−0.17	0.25	0.499
Laundry	−0.34	0.22	0.125
Dishes	0.17	0.18	0.341
Bathing self/child	−0.33	0.15	0.025*
Sweeping/mopping	0.06	0.16	0.688
Time since last handwashing with soap (log minutes)	0.39	0.17	0.023*

*P-value is below 0.05.
**P-value is below 0.01.

caused by the specific activities performed by the activity cohort. After observed handwashing with soap, all prescribed activities except for 'sitting' (control group) increased the levels of faecal bacteria on hands (Figure 1). The urination group had the highest mean increase in *E. coli* (3.8 log CFU per two hands), while the food preparation group showed the highest mean increase in enterococci (3.8 log CFU per two hands). Increases after activities that involve the use of soap and water were also documented. For example, washing dishes increased the levels of *E. coli* and enterococci by an average of 1.7 log CFU and 1.9 log CFU per two hands, respectively. In the 'sitting' group, *E. coli* showed an average reduction of 1.3 log CFU per two hands, while enterococci remained relatively constant.

Pathogen and faecal markers

Among baseline samples ($N = 78$) analysed for the presence of enterovirus as well as general and human-specific *Bacteroidales* genetic markers, seven (9%) were positive for enterovirus, 45 (58%) were positive for the general *Bacteroidales* marker, and two (3%) were positive for the human-specific *Bacteroidales* marker. No association was found between the levels of faecal indicator bacteria on hands, the presence of enterovirus and the general and human-specific *Bacteroidales* markers.

Among the subset of samples analysed for virulence genes found in EPEC, ETEC and EAEC, 33% (10 of 30) were positive for at least one pathogenic *E. coli* marker. Samples that tested positive for the presence of at least one pathogenic *E. coli* marker had 0.46 log CFU per two hands

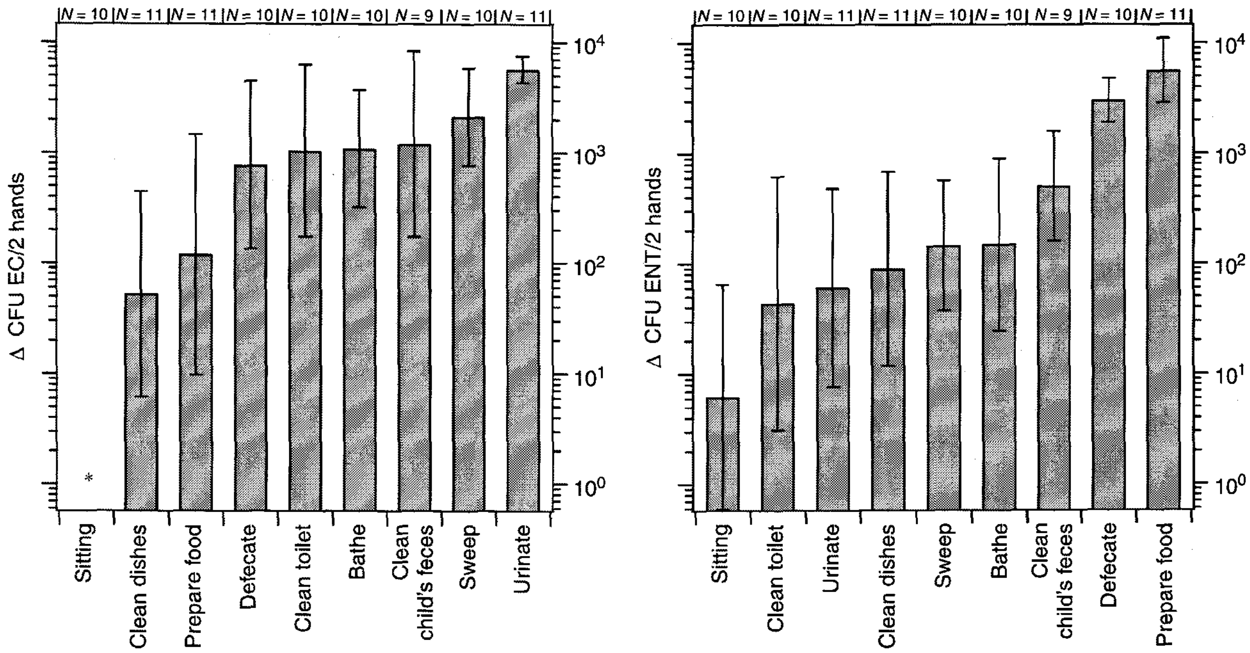

Figure 1 Geometric mean of change (Δ) in *Escherichia coli* (EC, left) and enterococci (ENT, right) per two hands attributed to specific observed activities. Error bars display SE of the log-transformed Δ FIB. *While all other activities increased EC, sitting *decreased* EC by a geometric mean of 20 CFU per two hands (SE 10).

more cultured *E. coli* (as measured by membrane filtration) than samples that tested negative (df = 28, $t = 2.19$, $P = 0.04$). Among the 12 baseline samples, 50% had at least one marker of pathogenic *E. coli* present on their hands, and each type of marker was present in 25% of samples. Among the 10 samples obtained after the mother had washed her hands with soap and water, 40% had at least one marker of pathogenic *E. coli*, and each marker was present in 20% of samples.

Discussion

Analysis of observational data paired with repeated microbiological hand rinse sampling reveals that many typical daily activities a Tanzanian mother undertakes can increase the amount of faecal indicator bacteria on her hands. Although enterococci were associated with handwashing behaviour in the expected direction, enterococci were also influenced by food preparation and leaving the home. These results indicate that, whereas faecal indicator bacterial levels on hands are correlated with hygiene behaviour, they are also influenced by many other actions that make faecal indicator bacteria highly variable indicators of hand hygiene behaviour.

Turbidity and pH of hand rinse samples were significantly associated with faecal indicator bacteria on hands. Higher levels of turbidity were associated with higher levels of faecal indicator bacteria, whereas lower pH levels were associated with higher levels of bacteria. Although the underlying mechanism behind the negative association between pH and faecal indicator organisms is unclear, washing hands with soap has been documented to increase skin pH (Gfatter *et al.* 1997). Further work should be carried out to validate pH and turbidity of hand rinses as indicators of handwashing behaviour.

Surprisingly, preparing food elevated faecal indicator bacterial levels on hands by similar magnitudes as visiting the toilet or cleaning up a child's faeces. Results from the observation cohort (in which enterococci were modelled as a function of observed activities) showed that mothers who prepared, ate or served food within 2 h of hand sampling had a mean of 0.84 more log enterococci per two hands than mothers who had not (Table 1). One explanation for this result is that produce and other foods may become contaminated with faeces during production, transport or handling in markets. The extent and source of faecal contamination on food in Dar es Salaam has not, to our knowledge, been studied. However, vegetables purchased from street markets in Ghana and Pakistan have been found to contain high levels of faecal bacteria, which could be the result of contamination through the use of wastewater for irrigation, application of manure to soil, post-harvest handling or during household storage (Amoah *et al.* 2007; Ensink *et al.* 2007).

It is notable that activities involving the use of soap, such as dishwashing and bathing, were associated with increases in faecal indicator bacteria on hands in the activity cohort. Enumerators documented the use of sand as a dish scrubbing agent in combination with soap one-third of the time. A sample of sand from the study community contained high levels of *E. coli* and enterococci (data not shown), suggesting it may be one source of hand contamination. Potential sources of faecal indicator bacteria while bathing could be the transfer of bacteria from other areas of the body to hands, or the presence of faecal indicator bacteria in stored water used for bathing.

The change in faecal indicator bacteria during specific activities was calculated assuming the fraction of bacteria eluted from a hand could be estimated with a constant value (f). However, it is possible that the fraction of bacteria removed by the hand rinse sampling method is variable and may be dependent on the level of faecal indicator bacteria on the hand and/or other factors. Regardless, a sensitivity analysis testing a range of values (0.25–0.75) for f does not change the trends observed in Figure 1, in which all activities except sitting increase the faecal indicator bacteria on hands.

Several findings in this study signify that *E. coli* and enterococci behave differently on hands. The model of hands as a function of activity data was significant for enterococci but not for *E. coli*, implying that *E. coli* on hands is a poor indicator of daily activities and hand hygiene behaviour. Possible explanations include differential rates of hand contamination with *E. coli* and enterococci from environmental surfaces, different concentrations in faeces and different survival rates on hands. *Escherichia coli* has been found to persist on the hands for shorter periods of time than faecal streptococci (Pinfold 1990a), which may also explain why *E. coli* levels were observed to decrease in the 'sitting' group in the activity cohort while enterococci levels were not.

Only 3% of hand rinse samples were positive for the *Bacteroidales* human-specific marker, while 58% were positive for the general marker. The difference may be attributed to non-human faecal sources of *Bacteroidales* or a low prevalence of human-specific *Bacteroidales* in faeces of the study population. Considering that human-specific *Bacteroidales* markers are present at concentrations of 100–1000 times lower than general *Bacteroidales* markers in faeces (Shanks *et al.* 2009), the concentration of human-specific *Bacteroidales* may be below the limit of detection in hand samples, while the general marker remains at a detectable concentration.

The occurrence of pathogenic *E. coli* markers in hand rinse samples was associated with higher cultured *E. coli* concentrations, but no relationship was found between faecal indicator bacterial levels and the presence of *Bacteroidales* or enterovirus. This implies that quantitative measures of faecal indicator bacteria are not able to predict the presence of select pathogens. However, the discovery of known pathogen and faecal markers on hands suggests hands are important vectors of disease among Tanzanian mothers. Further work on the persistence and ecology of the pathogens and indicator bacteria on skin in Africa is warranted.

The finding that contamination of hands with faecal indicator bacteria occurs during activities not traditionally viewed as faecal contamination events is unexpected. While it is unknown whether all the faecal indicator bacteria measured in this study originated from human faeces, the detection of genetic markers of faecal *Bacteroidales*, human-specific *Bacteroidales*, enterovirus and pathogenic *E. coli* indicates that at least a portion are faecal in origin. Almost all (99%) of the households in this study had access to a toilet facility, yet only 34% were connected to a septic tank. Detection of high levels of faecal indicator bacteria on hands after a broad range of daily activities suggests that extensive environmental faecal contamination likely persists in the community, however further research is needed to identify the sources of contamination. This study exposes how challenging personal and household hygiene can be for mothers in an environment with limited sanitation facilities, a situation typical in many developing countries.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Hand faecal contamination among Tanzanian mothers varies temporally and following household activities.

Table S1. Target gene, amplicon size, annealing temperatures, and primers used in detection of general *Bacteriodales*, human-specific *Bacteroidales*, EPEC, ETEC, EAEC, and enterovirus

Table S2. Participant characteristics

Table S3. Generalized estimating equations of log *E. coli* on hands, modeled as a function of observed activities and time since last handwashing with soap

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