American Journal of Infection Control 45 (2017) 228-33

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major Article

Handwashing, but how? Microbial effectiveness of existing handwashing practices in high-density suburbs of Harare, Zimbabwe

Max N.D. Friedrich MSc [a,*], Timothy R. Julian PhD [b], Andreas Kappler [c], Tamuka Nhiwatiwa PhD [d], Hans-Joachim Mosler [a]

[a] Department of Environmental Social Sciences, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland
[b] Department of Environmental Microbiology, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland
[c] Center for Applied Geoscience, University of Tübingen, Tübingen, Germany
[d] Department of Biology, University of Zimbabwe, Mount Pleasant, Harare, Zimbabwe

Key Words:
Recommendation
Efficacy
Procedure
Diarrhea
Steps

Background: Consistent domestic hand hygiene can reduce diarrhea-related morbidity and mortality and the spread of other communicable diseases. However, it remains uncertain which technique of handwashing is most effective and practicable during everyday life. The goal of this study is to determine how the handwashing technique, as performed in the daily life by the participants of this case study in Harare, Zimbabwe, influences microbial handwashing effectiveness.

Methods: Handwashing technique of 173 primary caregivers was observed in their homes and hand rinse samples were collected before and after handwashing. Samples were analyzed for *Escherichia coli* and total coliform concentrations. Generalized linear models were used to predict fecal hand contamination after washing from observed handwashing technique.

Results: Cleaning under fingernails, scrubbing the fingertips, using soap, and drying hands through rubbing on clothes or a clean towel statistically significantly reduced *E coli* contamination of hands after washing. Tap use, scrubbing fingertips, and rubbing hands on clothes to dry them statistically significantly reduced total coliform contamination.

Conclusions: Recommendations for effective and practicable domestic handwashing in Harare, Zimbabwe, should include performing specific handscrubbing steps (ie, cleaning under the fingernails and rubbing the fingertips), and soap and tap use. This calls for further research to develop behavior change interventions that explicitly promote effective handwashing technique at critical times.

Consistent hand hygiene can reduce diarrhea-related morbidity and mortality. Diarrhea is among the leading causes of childhood mortality worldwide.[1] Fischer Walker et al[2] estimated that in 2011, 700,000 children died of diarrhea, with highest rates in South East Asia and Africa. According to estimations by Prüss-Ustün et al,[3] 297.000 deaths were caused by inadequate hand hygiene worldwide during 2012. Handwashing at critical times, such as before eating, cooking, or other contact with food and after defecation and other contact with feces was shown to be among the most cost-effective methods to reduce diarrhea.[4-7] Despite its importance, handwashing with soap is only practiced by a small proportion of people worldwide.[4] This calls for effective handwashing promotion on a large scale.

Among others, the Centers for Disease Control and Prevention (CDC) and the World Health Organization provide recommendations on effective handwashing in health care settings.[8,9] For domestic handwashing, the CDC[10] recommends the following 5 steps:

1. Wet your hands with clean, running water (warm or cold), turn off the tap, and apply soap.
2. Lather your hands by rubbing them together with the soap. Be sure to lather the backs of your hands, between your fingers, and under your nails.
3. Scrub your hands for at least 20 seconds.
4. Rinse your hands well under clean, running water.
5. Dry your hands using a clean towel or air-dry them.

* Address correspondence to Max N.D. Friedrich, MSc, Department of Environmental Social Sciences, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Überlandstrasse 133, 8600 Dübendorf, Switzerland.

E-mail address: max.friedrich@eawag.ch (M.N.D. Friedrich).

This work was funded by the Swiss Agency for Development and Cooperation (SDC). SDC has not officially endorsed this publication and the views expressed may not represent the views of SDC.

Conflicts of interest: None to report.

0196-6553/© 2017 Association for Professionals in Infection Control and Epidemiology, Inc. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ajic.2016.06.035

However, the microbial effectiveness of the recommended steps is only partly substantiated. Further, it remains uncertain whether performing the steps throughout the daily routine is acceptable for potential participants of handwashing-promotion activities.

Comprehensive evidence to sustain the importance of the recommended steps is limited to soap use[11-14] and does not corroborate the remaining steps. To our knowledge, there are no studies corroborating the first step's suggestion for running water: No studies have compared the effectiveness of handwashing with running water versus, for example, stored water. With regard to characteristics of handwashing water, field experiments suggest that increased water volume and quality of handwashing water are associated with cleaner hands after washing.[15] Further, the influence of thoroughness of handwashing, described by both length[12,16-18] and scrubbing steps[19] is uncertain. Evidence on which hand-drying technique is most effective are mixed[20-22] and recontamination of hands from contaminated clothes is likely.[23] None of the existing studies evaluate the relative importance of different handwashing steps. Further, most presented findings originate from laboratory or field experiments that compared prespecified handwashing regimens, in which singular handwashing steps were manipulated while the remaining handwashing technique remained constant.[11,12,16,19] The studies therefore do not represent handwashing as performed by community members in their daily life, which some authors have suggested should be tested.[12,24]

Handwashing campaigns should promote a technique of handwashing that is both effective in the local context and acceptable for the target population. Everyday life compliance with washing hands according to complex guidelines is assumed to be low.[18,24] Particularly in developing countries, the CDC guidelines may be difficult to follow because running water from a tap and a clean towel, for instance, are often not available.[25] In addition, local customs may suggest different handwashing procedures, such as in Zimbabwe where hands are traditionally moistened and rinsed in a bowl of water.[26] As a consequence, investigating which handwashing steps are already in practice in the target population and determining their effectiveness in the context where they are usually performed is needed to decide which handwashing technique should be promoted.

Taken together, there are substantial gaps in the understanding of which handwashing technique to promote to achieve microbial effective domestic handwashing in a specific target population. The goal of the present study is to determine how the handwashing technique, as performed in the daily life by the participants of this case study in Harare, Zimbabwe, influences handwashing effectiveness. Based on the findings, substantiated and parsimonious recommendations for effective handwashing in the target population are provided.

MATERIALS AND METHODS

Participants

This study was implemented during June and July 2014 in 10 high-density and low-income suburbs of Harare, Zimbabwe. One working day before data collection, participants were recruited through random route sampling by selecting every fifth household starting from junctions in the study area. Because this study was part of a larger study, households needed to have at least 1 child attending the local primary school to be included in the sampling frame. Within each household, the primary caregiver was selected for the study and informed written consent was obtained. Nonresponding, ineligible, and refusing households were replaced by the fifth-next household on the sampling route. In total, 198 primary care givers were sampled.

Enumerator training

Before data collection, enumerators were enrolled in a 1-week training on sampling, observation, and interviewing techniques. To maximize standardization in the enumerators' assessment of handwashing techniques, enumerators performed the different components of handwashing themselves and practiced observation of each other's handwashing technique under supervision during the training. During a second training week, enumerators practiced data collection in the field and performed handwashing observations under supervision in at least 1 household before the actual data collection.

Data collection

Microbial contamination of hands was measured using hand rinse samples as previously reported.[27] The data collector randomly selected the first hand to be sampled through the random function of OpenDataKit software (Department of Computer Science and Engineering, University of Washington, Seattle, WA) on a tablet computer.[28] The selected hand of the participant was placed in a 2,040 mL sterile sampling bag (NASCO Corp, Fort Atkinson, WI) filled with 350 mL bottled water containing 17.5 mg/L sodium thiosulfate. Sodium thiosulfate had been added to inactivate residual chlorine potentially present in the water. The bag was fastened around the participant's wrist with a flexible rubber strap. The participant's hand was massaged in a standardized way. First, the palm of the hand, excluding fingers, was massaged for 10 seconds. Then, for each finger, the palm and back of the finger were simultaneously massaged for 5 seconds, both sides of the finger were simultaneously massaged for 5 seconds, the tip of the finger was massaged for 5 seconds, and the webbing to the subsequent finger for 5 seconds. Finally the back of the hand was massaged for 10 seconds. The participant's hand was withdrawn from the bag and the bag was closed and immediately placed in a cooler box with ice. The participant's hand was dried with a paper towel. Enumerators wore new nonsterile gloves for each hand sampling.

Subsequently, the participant was requested to wash hands in the way the participant would usually do either "before handling food" or "after contact with feces." The prompt concerning which of the 2 critical moments the enumerator stated was determined through the OpenDataKit random function. The respondent was explicitly reminded to demonstrate the way he or she would usually wash hands in such occasions. Structured observation of the demonstrated handwashing technique was performed while the total time that the respondent washed hands was determined with the stopwatch function of the enumerator's wristwatch. Handwashing steps observed included method of moistening hands, soap or other detergent use, performed scrubbing steps, method of rinsing hands, and way of drying hands (Table 1).

The second hand rinse sample was taken immediately after the handwashing demonstration from the hand that had not yet been sampled. The same procedure as described for the first sample was applied. After the second sampling, the enumerator recorded the handwashing observation data on the tablet computer.

The sociodemographic characteristics of participants were subsequently collected in a standardized face-to-face interview. The questionnaire had been developed in English, translated into the local Shona language, and retranslated into English to reduce risk of potential translation mistakes. It was programmed with OpenDataKit and filled on tablet computers. Spot-check observations regarding the presence of separate handwashing facilities for food- and stool-related handwashing, presence of soap and water at these locations, and type of the device to dispense water were performed at the end of each household visit.

Table 1
Observed handwashing technique, categorical characteristics (N = 173)

Handwashing technique	n	%
Moistening/rinsing		
Used tap*	72	42
Poured water on hands	29	17
Dipped hands into water	72	42
Soap use		
Did not use soap*	91	53
Used soap	82	47
Handscrubbing		
Scrubbed the palm	173	100
Scrubbed back	160	92
Scrubbed between fingers	102	59
Scrubbed under nails	46	27
Scrubbed fingertips	50	29
Scrubbing time >20 sec	107	62
Drying		
Air dried*	134	77
Rubbed hands on clothes	23	13
Used clean towel	7	4
Used dirty towel	9	5

*Used as reference category for multivariate models.

A subset of the study participants had been surveyed during a 3-hour structured handwashing observation on the same day before hand sampling.

Indicator organisms

Fecal indicator bacteria (total coliforms and *Escherichia coli)* were used as objective measures of handwashing effectiveness. Total coliforms are bacteria defined by their ability to be cultured in selective media for gram-negative microorganisms containing lactose when incubated at 35°C-37°C. Total coliforms are ubiquitous in the environment and are not necessarily associated with sanitary risks. However, total coliforms are used as an indicator of process effectiveness comparing concentrations before and after treatment (eg, before and after handwashing). *E coli* are a species of bacteria found in the gut of warm-blooded animals and are a subset of total coliforms. Although many strains of *E coli* are pathogenic, most are harmless. Hand hygiene studies conducted in the field rely on fecal indicator bacteria concentrations on hands to quantify fecal hand contamination and handwashing effectiveness.[12,21,29,30]

To evaluate handwashing effectiveness, both the log difference of hand contamination before and after washing[12,27] and the log hand contamination after washing[11,15] have been used in previous studies. In this study, hand contamination after washing was chosen as the outcome variable because hand contamination has been shown to be directly associated with diarrhea.[29,31] In addition, using log differences would not differentiate between reductions of different magnitudes (eg, reduction from 3-2 log CFU/hand amounting to 900 CFU/hand would be modeled in the same way as a reduction from 2-1 log CFU/hand amounting to 90 CFU/hand).

Laboratory procedures

Samples were cooled with ice and transported to the laboratory of the Department of Biology, University of Zimbabwe. They were processed by 2 trained students within 6 hours after collection. Samples were processed in triplicate and were analyzed for numbers of *E coli* and total coliform bacteria. Portions of 100 mL and 10 mL of each sample, representing 2/7ths and 2/70ths of the total sample collected, respectively, were passed through a 0.45-µm, 47-mm-diameter cellulose filter (Merck Millipore, Darmstadt, Germany) and placed on compact dry EC media plates (Nissui Pharmaceuticals, Tokyo, Japan). Before filtering a new sample, the filter unit was flamed with 80% ethanol, left for few minutes to cool down completely, and rinsed with bottled water containing 17.5 mg/L sodium thiosulfate. For the prewash sample, additional media plates were directly inoculated with 1 mL sampling solution because, compared with the postwash samples, higher contamination was expected. Plates were incubated for 24 ± 0.5 hours at 37°C ± 1°C. *E coli* and total coliforms were counted per manufacturer's instructions. At least 1 blank sample was run per day of data processing resulting in a total of 28 blank samples over the course of the study.

Data processing and statistical analyses

From the initial 198 participants who were sampled, 25 participants had to be excluded because of violations of the sampling and processing protocol, such as storage time of the samples exceeding 6 hours. The data of the remaining 173 participants were processed as follows. To obtain the value for each replicate, the sum of detected colony forming units across all plates of this replicate was divided by the total amount of sampling solution used for the respective replicate. Plates exceeding the maximum number of 250 CFU/plate were not countable and excluded. If no colonies were detected on any plate, the lower detection limit (3.2 CFU/hand) was inserted. The upper detection limits for the prewash and postwash samples were 87,500 CFU/hand and 8,750 CFU/hand, respectively. Of each sample, the mean of the 3 replicates was taken. For 17 of 331 total samples, only duplicates were available, due to processing mistakes.

Paired-samples *t* tests were performed to test significance of the change in $\log_{10}$ colony forming units per hand of *E coli* and total coliform bacteria during handwashing. Fecal indicator bacteria per hand after handwashing were modeled as a function of the performed handwashing technique (eg, way of moistening hands and way of scrubbing hands), the prewash hand contamination and the order of hand sampling. A generalized linear model, with 10 log as a link function, a negative binomial distribution, and robust estimates of the parameters' standard errors was used. Categorical predictors were entered as dummy variables as displayed in Table 1. Further, the initial hand contamination before washing was modeled as continuous predictors. To obtain unbiased parameter estimates, outliers with log colony forming units larger than 4× the standard deviation were removed from the models. For the model predicting *E coli* counts after washing, the outliers included 6 participants (<4% of the total sample). For the model predicting total coliform counts after washing, 14 participants (8% of the total sample) were excluded. Therefore, the model results are restricted to individuals who achieved hand contamination <3.2 log CFU *E coli* per hand or 3.6 log CFU total coliforms per hand after handwashing.

Ethical approval

The study protocol was reviewed and approved by the Medical Research Council of Zimbabwe, the Research Council of Zimbabwe, and the Ethical Review Board of the University of Zürich.

RESULTS

Participants and study area

One hundred seventy-one participants (99%) were women and 2 participants (1%) were men. On average, the participants had attended 10 ± 2.7 years of formal education and were aged 37 ± 11.5 years. The average household size of participants amounted to 5.6 ± 1.8 household members and the average monthly household income was $341 ± $290.

Table 2
Generalized linear model of *Escherichia coli* and total coliform $\log_{10}$ colony forming units per hand after handwashing, modeled as a function of the performed handwashing technique

Handwashing technique	*E coli*			Total coliforms		
		95% Confidence interval†			95% Confidence interval†	
	B	Lower	Upper	*B*	Lower	Upper
Intercept	**1.45****	**0.37**	**2.53**	**2.16****	**0.91**	**3.40**
Moistening/rinsing						
Poured water on hands	0.16	−0.38	0.70	**0.77****	**0.23**	**1.32**
Dipped hands into water	**0.82*****	**0.36**	**1.28**	**0.76****	**0.20**	**1.32**
Soap use	**−0.87****	**−1.36**	**−0.37**	0.06	−0.48	0.60
Handscrubbing						
Scrubbed back	−0.11	−0.89	0.66	0.88	−0.14	1.90
Scrubbed between fingers	−0.77	−1.77	0.22	1.26	−0.04	2.56
Scrubbed under nails	**−3.87*****	**−5.46**	**−2.28**	−0.98	−2.57	0.61
Scrubbed fingertips	**−3.62*****	**−5.20**	**−2.04**	**−1.41****	**−2.21**	**−0.60**
Interaction: Handscrubbing						
Scrubbed back × scrubbed between fingers	0.66	−0.43	1.75	−1.22	−2.61	0.17
Scrubbed back × scrubbed under nails	**2.50*****	**1.29**	**3.71**	0.82	−0.47	2.11
Scrubbed back × scrubbed fingertips	**3.05*****	**1.85**	**4.25**	‡	‡	‡
Scrubbed between fingers × scrubbed under nails	0.98	−0.21	2.17	−0.84	−1.93	0.25
Scrubbed between fingers × scrubbed fingertips	0.80	−0.30	1.90	0.98	0.00	1.97
Scrubbed under nails × scrubbed fingertips	0.02	−0.98	1.02	**1.26***	**0.20**	**2.32**
Scrubbing time >20 sec	0.54	−0.08	1.15	0.58	−0.13	1.29
Drying						
Rubbed hands on clothes	**−0.85****	**−1.39**	**−0.31**	**−0.61***	**−1.13**	**−0.09**
Used clean towel	**−1.35****	**−2.25**	**−0.44**	−0.37	−1.33	0.59
Used dirty towel	−0.08	−0.89	0.73	0.83	−0.53	2.18
Total wash time (seconds)	0.00	−0.02	0.02	0.00	−0.03	0.02
Hand contamination before washing	**1.22*****	**0.94**	**1.50**	**0.85*****	**0.64**	**1.06**
Right hand sampled first	0.42	−0.04	0.88	0.05	−0.41	0.51

NOTE. Model *E coli* likelihood ratio, $\chi^2(20) = 242.89$; $P < .001$, and model *total coliforms* likelihood ratio, $\chi^2(19) = 147.44$, $P < .001$.
†Wald confidence interval (*B*).
‡No parameter estimate computed because not all combinations of rubbing back of hands × rubbing fingertips were observed.
*$P < .05$.
**$P < .01$.
***$P < .001$.

Observed handwashing infrastructure

One hundred fifty-six participants (90%) showed a specific place for handwashing. One hundred twenty-eight participants (74%) showed separate handwashing places for food- and stool-related handwashing. In 143 households (83%), water was present at 1 or more handwashing locations. One hundred three households (60%) had a place for handwashing with a water tap; most (86 households, or 83% of those with taps) had running tap water at the time of data collection. In 111 households (64%), soap was present at 1 or more handwashing locations.

Blanks

No *E coli* or total coliform colonies appeared on any of the blank samples.

Hand contamination before and after washing

In the prewash sample the average $\log_{10}$ CFU *E coli* per hand was 1.4 ± 0.9. The postwash samples yielded, on average, 1.2 ± 0.8 $\log_{10}$ CFU/hand. The reduction was statistically significant ($t[172] = 4.28$; $P < .001$). The mean hand contamination with total coliform bacteria was reduced by 0.3 $\log_{10}$ CFU/hand from 2.5 ± 1.0 $\log_{10}$ CFU/hand in the prewash sample to 2.2 ± 0.9 $\log_{10}$ CFU/hand in the postwash sample ($t[172] = 4.28$; $P < .001$).

Observed handwashing technique

Table 1 presents the observed handwashing technique of participants.

From 94 participants, the right hand was sampled first and from 79 participants the left hand was sampled first. The average time spent moistening, scrubbing, and rinsing hands amounted to 25.6 ± 14.9 seconds (median, 23.0 seconds) with an interquartile range of 16-32 seconds.

Effectiveness of handwashing technique

Results of a generalized linear model predicting the *E coli* and total coliform contamination of hands after washing based on the performed handwashing steps are presented in Table 2. For *E coli,* moistening and rinsing hands by dipping them into a vessel with water was statistically significantly associated with more contaminated hands after washing. Scrubbing the fingertips and under the fingernails led to significantly lower contamination of hands after washing. The positive and significant value of the interaction terms for scrubbing the back of the hands and under the nails and scrubbing the back of the hands and the fingertips indicate that the individual effect of each step is decreased when both are performed. Soap use improved the overall effectiveness of handwashing. The data further show that drying hands by rubbing them on the clothes or using a clean towel for hand drying was associated with cleaner hands after washing.

With regard to total coliform contamination after washing, moistening hands by pouring water from a vessel and dipping hands into a vessel led to higher hand contamination than moistening and rinsing hands under a tap. Soap use, in contrast to the results for *E coli*, was not statistically significantly related to total coliform contamination. Among the scrubbing steps, scrubbing the fingertips was associated with cleaner hands after washing. Like in the previous model for *E coli*, drying hands by rubbing them on clothes led to lower contamination than air drying. However, using a clean towel was not associated with cleaner hands after washing.

Some trends consistent in both models were observed. For example, neither the total time spent for handwashing, nor scrubbing hands for more than 20 seconds, as it is recommended by CDC, was related to cleaner hands after washing. In addition, similar effects of moistening hands by dipping them into a vessel, scrubbing the fingertips, and drying hands on clothes were observed in both models. Also, the contamination before washing was a significant predictor of hand contamination after washing in both models.

DISCUSSION

The goal of the present study was to determine which steps of handwashing, as performed in the everyday life in suburbs of Harare, Zimbabwe, matter most to yield clean hands and to provide parsimonious recommendations for effective domestic handwashing in the study population. In a cross-sectional survey, the handwashing technique of primary caregivers in Harare was observed and its influence on hand contamination after washing was quantified. This study shows that recommendations for effective hand hygiene should include moistening hands under a tap; soap use; and performing specific scrubbing steps, including scrubbing under the fingernails and scrubbing the fingertips. Furthermore, the study suggests that inclusion of a minimum wash or scrubbing time may complicate recommendations without providing any additional bacterial removal. These findings from suburbs of Harare, Zimbabwe, demonstrate that handwashing technique influences microbial effectiveness. Future handwashing behavior change interventions should target both handwashing frequency and technique. The results further suggest a critical evaluation of existing handwashing recommendations in low- and middle-income countries.

Moistening hands under a tap led to cleaner hands than dipping hands into a vessel. For total coliforms, tap use was also more effective than manually pouring water on hands. Several mechanisms of action may lead to this effect. First, tap water was always from a fixed tap supplied by municipal water. This water might be less contaminated than stored water that was used when manually pouring water on hands or dipping hands into a vessel.[29,32-34] As Hoque et al[15] suggested, using contaminated handwashing water may result in more fecal contamination on hands than using clean water. Different levels of water contamination for *E coli* and total coliforms may explain the observed differences between the indicator organisms. Moistening hands by dipping them into the water, which is later used for rinsing, might more strongly contaminate the water with the more transient *E coli* bacteria than with total coliforms. Second, using a tap allows handscrubbing during hand moistening and rinsing, which may lead to additional removal of germs at this time. Third, tap water was available in larger quantity than stored water. This is likely to have prompted tap users to use more water, which may have further reduced hand contamination. Findings from a field experiment by Hoque et al[15] support this hypothesis showing that individuals who washed hands using 2 L water had lower loads of fecal indicator bacteria on hands after washing than individuals who used only 0.5 L.[15]

Specific handscrubbing steps such as scrubbing under the fingernails and scrubbing the fingertips make handwashing more effective. We demonstrated reduced *E coli* counts after handwashing when these steps were performed and reduced total coliform counts when the fingertips were scrubbed. Higher contamination of fingernails with *E coli* than with total coliforms may account for the different effects of scrubbing under the nails. To our knowledge this is the first time that the effects of specific scrubbing steps were investigated. Because these steps do not require additional material, such as soap or a water tap, our findings highlight an opportunity for handwashing campaigns to increase handwashing effectiveness without providing additional hardware.

Neither scrubbing hands for at least 20 seconds or total handwashing time were associated with hand cleanliness after washing. This is in contrast with the CDC recommendation but corroborates previous experimental findings.[12,16,17] This result is relevant for future handwashing promotion because it is doubtful whether people comply with complex recommendations that include minimum handwashing times.[24] In contrast, recommending specific handwashing steps without a time limit might reduce complexity and increase compliance.

Using soap led to significantly less *E coli* counts on hands than washing hands with water only. Controlling for other handwashing steps that were usually performed by this study's participants, this corroborates the importance of soap, previously demonstrated in experimental trials.[11,12] However, it is important to note that the effect of soap was less than one-third of that of rubbing under the nails and scrubbing the fingertips. This finding highlights the importance to perform specific handscrubbing steps. Further, soap did not affect total coliform contamination. Being ubiquitous in the environment total coliforms may be part of the resident bacteria on hands and more difficult to remove than *E coli*.

Drying hands on clothes led to less *E coli* and total coliforms on hands. Using a clean towel led to less *E coli* bacteria on hands than air drying. Rubbing hands on a towel or on clothes may have physically removed bacteria from hands as hypothesized by Huang et al.[20] This questions the CDC recommendations that also promote air drying following handwashing.

Limitations

Individuals were not randomized to specific handwashing regimens, which limits the given recommendations to handwashing steps already in practice in the study community. However, this design allowed us to assess the importance of handwashing steps as performed in the usual way and give handwashing recommendations that are adapted to the real life of the study population. Furthermore, the results show that, except for hand drying with a clean and a dirty towel, all steps that we aimed to test were performed by a sufficient number of participants to evaluate them. More studies at other sites are required to generalize the given recommendations for effective handwashing.

Participants were directly observed by the enumerators during the handwashing demonstration that may have prompted participants to wash hands differently than they normally would.[35] To minimize these effects, participants were explicitly reminded to wash hands in the usual way, that all information that was collected from them was handled confidentially, and that they would help their community most if they washed hands in the usual way. However, even if the handwashing demonstration had been biased, it would not have affected the relationship between the performed handwashing technique and its effectiveness.

E coli and total coliform concentrations obtained from culture-based methods are imperfect indicators of hand contamination. We included in our results all observable bacterial colonies present on the compact dry plates that fit the manufacturer's description. We may, therefore, have overestimated fecal bacterial contamination by including false positives with atypical morphology that nevertheless fit the manufacturer's description. Although Julian et al[36] observed low false positive rates for *E coli* on hands in Bangladesh as measured using the Colilert assay (which, like compact dry plates, relies on the presence of the β-galactosidase enzyme for *E coli* identification), we did not attempt to confirm colonies isolated in this study.

Conclusions

This study shows that the handwashing technique is paramount for handwashing to effectively decontaminate hands. Worldwide, huge efforts are made to promote frequent handwashing with soap at critical times. Our findings raise the need to extend the focus of handwashing interventions to promoting effective techniques that include moistening hands with running water and performing specific handscrubbing steps. Field studies in other settings are needed to corroborate these results and further investigate the influence of hand drying methods. Handwashing steps as recommended by CDC are already in practice by some individuals of the survey population, which suggests that achieving uptake of the recommended technique by a larger share of the population can be a realistic aim of behavior change interventions. Additional research is needed to understand the behavioral determinants that drive people to apply effective handwashing techniques. This should support the design of interventions that make handwashing as effective as possible where the disease burden is high and resources are limited.

Acknowledgments

The authors thank Frederik Hammes for his advice on the laboratory procedures; Pablo Streich, Innocent Hove, Ellen Struve, and Karin Stögerer for their laboratory contributions; our field coordinators Belladonnah Muzavazi and Eustace Sangoya; the enumerator team; and all study participants in Harare.

References

1. Rudan I, El Arifeen S, Black RE, Campbell H. Childhood pneumonia and diarrhoea: setting our priorities right. Lancet Infect Dis 2007;7:56-61.
2. Fischer Walker CL, Rudan I, Liu L, Nair H, Theodoratou E, Bhutta ZA, et al. Global burden of childhood pneumonia and diarrhoea. Lancet 2013;381:1405-16.
3. Prüss-Ustün A, Bartram J, Clasen T, Colford JM, Cumming O, Curtis V, et al. Burden of disease from inadequate water, sanitation and hygiene in low- and middle-income settings: a retrospective analysis of data from 145 countries. Trop Med Int Health 2014;19:894-905.
4. Freeman MC, Stocks ME, Cumming O, Jeandron A, Higgins JPT, Wolf J, et al. Hygiene and health: systematic review of handwashing practices worldwide and update of health effects. Trop Med Int Health 2014;19:906-16.
5. Curtis V, Cairncross S. Effect of washing hands with soap on diarrhoea risk in the community: a systematic review. Lancet Infect Dis 2003;3:275-81.
6. Feachem RG. Interventions for the control of diarrhoeal diseases among young children: promotion of personal and domestic hygiene. Bull World Health Organ 1984;62:467-76.
7. Borghi J, Guinness L, Ouedraogo J, Curtis V. Is hygiene promotion cost-effective? A case study in Burkina Faso. Trop Med Int Health 2002;7:960-9.
8. Centers for Disease Control and Prevention. Guideline for hand hygiene in healthcare settings: Recommendations of the healthcare infection control practices advisory committee and the HICPAC/SHEA/APIC/IDSA hand hygiene task force. Atlanta: Center for Disease Control and Prevention; 2002.
9. World Health Organisation. WHO guidelines on hand hygiene in health care. Geneva: World Health Organisation; 2009.
10. Centers for Disease Control and Prevention. When and how to wash your hands. Available from: http://www.cdc.gov/handwashing/when-how-handwashing.html. October 2, 2015.
11. Burton M, Cobb E, Donachie P, Judah G, Curtis V, Schmidt W-P. The effect of handwashing with water or soap on bacterial contamination of hands. Int J Environ Res Public Health 2011;8:97-104.
12. Amin N, Pickering AJ, Ram PK, Unicomb L, Najnin N, Homaira N, et al. Microbiological evaluation of the efficacy of soapy water to clean hands: a randomized, non-inferiority field trial. Am J Trop Med Hyg 2014;91:415-23.
13. Luby S, Agboatwalla M, Raza A, Sobel J, Mintz E, Baier K, et al. Microbiologic effectiveness of hand washing with soap in an urban squatter settlement, Karachi, Pakistan. Epidemiol Infect 2001;127:237-44.
14. Sickbert-Bennett EE, Weber DJ, Gergen-Teague MF, Rutala WA. The effects of test variables on the efficacy of hand hygiene agents. Am J Infect Control 2004;32:69-83.
15. Hoque BA, Mahalanabis D, Alam MJ, Islam MS. Post-defecation handwashing in Bangladesh: practice and efficiency perspectives. Public Health 1995;109:15-24.
16. Lucet JC, Rigaud MP, Mentre F, Kassis N, Deblangy C, Andremont A, et al. Hand contamination before and after different hand hygiene techniques: a randomized clinical trial. J Hosp Infect 2002;50:276-80.
17. Fuls JL, Rodgers ND, Fischler GE, Howard JM, Patel M, Weidner PL, et al. Alternative hand contamination technique to compare the activities of antimicrobial and nonantimicrobial soaps under different test conditions. Appl Environ Microbiol 2008;74:3739-44.
18. Conover DM, Gibson KE. A review of methods for the evaluation of handwashing efficacy. Food Control 2016;63:53-64.
19. Lin C-M, Wu F-M, Kim H-K, Doyle MP, Michaels BS, Williams LK. A comparison of hand washing techniques to remove *Escherichia coli* and Caliciviruses under natural or artificial fingernails. J Food Prot 2003;66:2296-301.
20. Huang C, Ma W, Stack S. The hygienic efficacy of different hand-drying methods: a review of the evidence. Mayo Clin Proc 2012;87:791-8.
21. Hoque B, Mahalanabis D, Pelto B, Alam M. Research methodology for developing efficient handwashing options: an example from Bangladesh. J Trop Med Hyg 1995;98:469-75.
22. Gustafson DR, Vetter EA, Larson DR, Ilstrup DM, Maker MD, Thompson RL, et al. Effects of 4 hand-drying methods for removing bacteria from washed hands: a randomized trial. Mayo Clin Proc 2000;75:705-8.
23. Gil AI, Lanata CF, Hartinger SM, Mäusezahl D, Padilla B, Ochoa TJ, et al. Fecal contamination of food, water, hands, and kitchen utensils at the household level in rural areas of Peru. J Environ Health 2014;76:102-6.
24. Bloomfield SF, Aiello AE, Cookson B, O'Boyle C, Larson EL. The effectiveness of hand hygiene procedures in reducing the risks of infections in home and community settings including handwashing and alcohol-based hand sanitizers. Am J Infect Control 2007;35:27-64.
25. Sandhu MP, Goodnight J. Community handwashing guide: utilizing available resources to initiate a handwashing intervention. World Med Health Policy 2014;6:39-50.
26. Kaltenthaler E, Waterman R, Cross P. Faecal indicator bacteria on the hands and the effectiveness of hand-washing in Zimbabwe. J Trop Med Hyg 1991;94:358.
27. Pickering AJ, Boehm AB, Mwanjali M, Davis J. Efficacy of waterless hand hygiene compared with handwashing with soap: a field study in Dar es Salaam, Tanzania. Am J Trop Med Hyg 2010;82:270-8.
28. Hartung C, Lerer A, Anokwa Y, Tseng C, Brunette W, Borriello G. Open data kit: Tools to build information services for developing regions. Proceedings of the 4th ACM/IEEE International Conference on Information and Communication Technologies and Development. London, United Kingdom: ACM; 2010. p. 1-12.
29. Pickering AJ, Davis J, Walters SP, Horak HM, Keymer DP, Mushi D, et al. Hands, water, and health: fecal contamination in Tanzanian communities with improved, non-networked water supplies. Environ Sci Technol 2010;44:3267-72.
30. Pickering AJ, Julian TR, Mamuya S, Boehm AB, Davis J. Bacterial hand contamination among Tanzanian mothers varies temporally and following household activities. Trop Med Int Health 2011;16:233-9.
31. Pinfold JV, Horan NJ. Measuring the effect of a hygiene behaviour intervention by indicators of behaviour and diarrhoeal disease. Trans R Soc Trop Med Hyg 1996;90:366-71.
32. Genthe B, Strauss N, Seager J, Vundule C, Maforah F, Kfir R. The effect of type of water supply on water quality in a developing community in South Africa. Water Sci Technol 1997;35:35-40.
33. Palit A, Batabyal P, Kanungo S, Sur D. In-house contamination of potable water in urban slum of Kolkata, India: a possible transmission route of diarrhea. Water Sci Technol 2012;66:299-303.
34. Levy K, Nelson KL, Hubbard A, Eisenberg JN. Following the water: a controlled study of drinking water storage in northern coastal Ecuador. Environ Health Perspect 2008;116:1533-40.
35. Kohli E, Ptak J, Smith R, Taylor E, Talbot EA, Kirldand KB. Variability in the Hawthorne effect with regard to hand hygiene performance in high- and low-performing inpatient care units. Infect Control Hosp Epidemiol 2009;30:222-5.
36. Julian TR, Islam MA, Pickering AJ, Roy S, Fuhrmeister ER, Ercumen A, et al. Genotypic and phenotypic characterization of *Escherichia coli* isolates from feces, hands, and soils in rural Bangladesh via the Colilert Quanti-tray System. Appl Environ Microbiol 2015;81:1735-43.