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Microbiological Evaluation of the Efficacy of Soapy Water to Clean Hands: A Randomized, Non-Inferiority Field Trial

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Abstract. We conducted a randomized, non-inferiority field trial in urban Dhaka, Bangladesh among mothers to compare microbial efficacy of soapy water (30 g powdered detergent in 1.5 L water) with bar soap and water alone. Fieldworkers collected hand rinse samples before and after the following washing regimens: scrubbing with soapy water for 15 and 30 seconds; scrubbing with bar soap for 15 and 30 seconds; and scrubbing with water alone for 15 seconds. Soapy water and bar soap removed thermotolerant coliforms similarly after washing for 15 seconds (mean $\log_{10}$ reduction = 0.7 colony-forming units [CFU], $P < 0.001$ for soapy water; mean $\log_{10}$ reduction = 0.6 CFU, $P = 0.001$ for bar soap). Increasing scrubbing time to 30 seconds did not improve removal ($P > 0.05$). Scrubbing hands with water alone also reduced thermotolerant coliforms (mean $\log_{10}$ reduction = 0.3 CFU, $P = 0.046$) but was less efficacious than scrubbing hands with soapy water. Soapy water is an inexpensive and microbiologically effective cleansing agent to improve handwashing among households with vulnerable children.

INTRODUCTION

Each year, approximately 600,000 children < 5 years old, mostly from low-income countries, die of diarrhea.[1] Handwashing with soap after defecation and handling feces and before preparing and eating food can reduce the risk of diarrhea.[2–4] In both rural and urban communities of Bangladesh, people rarely wash their hands with soap at recommended times.[5,6] In a study among rural Bangladeshi caregivers, fewer than 1% used soap and water for handwashing before eating and/or feeding a child, and only 33% of caregivers and 14% of all household members were observed washing both hands with soap after defecation.[5]

Barriers to washing hands with soap in low-income communities include the high cost of soap relative to household income, the concern that soap left out at a common handwashing place could be stolen, and the concern that children could play with or waste the bar soap.[7–11] A study from 68 subdistricts of Bangladesh suggested that rural residents who live in households with either water or soap at the handwashing place were two times as likely to wash both hands with soap after contact with feces as those residents who did not have soap or water conveniently available.[12] In addition, Bangladeshi urban households from the wealthiest quintile were more likely to have soap consistently at handwashing stations (odds ratio [OR] = 1.9, 95% confidence interval [95% CI] = 1.4–2.4) and wash their hands with soap at critical times (adjusted OR [OR_{adj}] = 1.4, 95% CI = 1.1–1.7).[13] A number of research studies from Bangladesh have shown that the cost of soap is a barrier to its use.[7–11] These findings suggest that overcoming economic barriers to maintaining access to soap in the home could increase handwashing frequency.

Soapy water is a mixture of powder detergent in water, previously introduced as a handwashing agent in Kenya and Peru in 2008.[14,15] Soapy water is currently being piloted in low-income rural[16] and urban[17] communities in Bangladesh[18] for acceptability and feasibility. To make soapy water, 30 g powdered detergent (Wheel, Unilever, Dhaka, Bangladesh) is mixed in any 1.5 L container, such as a reused water/soda/juice bottle. Preliminary qualitative research suggests that soapy water is popular because of its low cost and ease of preparation.[16] In Bangladesh, a 30 g sachet of powder laundry detergent costs US$0.03 compared with a common bar of soap (Lux, Unilever, Dhaka, Bangladesh), which costs US$0.35. The cost of freshly prepared soapy water is US$0.09 (detergent US$0.03 + plastic bottle US$0.06), and the cost of refilling an existing bottle is only US$0.03. Therefore, the lower cost of soapy water compared with bar soap and the fact that it can be kept in a reused plastic bottle may allow households to ensure the availability of a cleansing agent for handwashing at home. In addition, soapy water may be less likely to be stolen than bar soap because of its low cost.[18]

However, there are no data on the microbial efficacy of handwashing with soapy water in field settings. In a low-income urban area in Dhaka, Bangladesh, we conducted a randomized, non-inferiority field trial to compare the efficacy of soapy water with the efficacy of bar soap and water alone for removing thermotolerant coliforms and *Clostridium perfringens* from hands. We also compared the efficacy of soapy water and bar soap using two different hand scrubbing times (15 and 30 seconds).

METHODS

A non-inferiority trial seeks to determine whether a new treatment (soapy water) is not worse than a reference treatment (bar soap) by more than an acceptable amount.[19]

Selection and enrolment of respondents. We conducted this study from July to September 2011 in the Mirpur area of urban Dhaka among mothers with at least one child < 5 years old recruited from an ongoing observational study unrelated to hand hygiene.[20] The study area was selected for the handwashing trial, because this area represents the other low-income urban slums of Bangladesh with high levels of environmental contamination. Using the Microsoft Excel random number generator, one of the investigators (N.A.)

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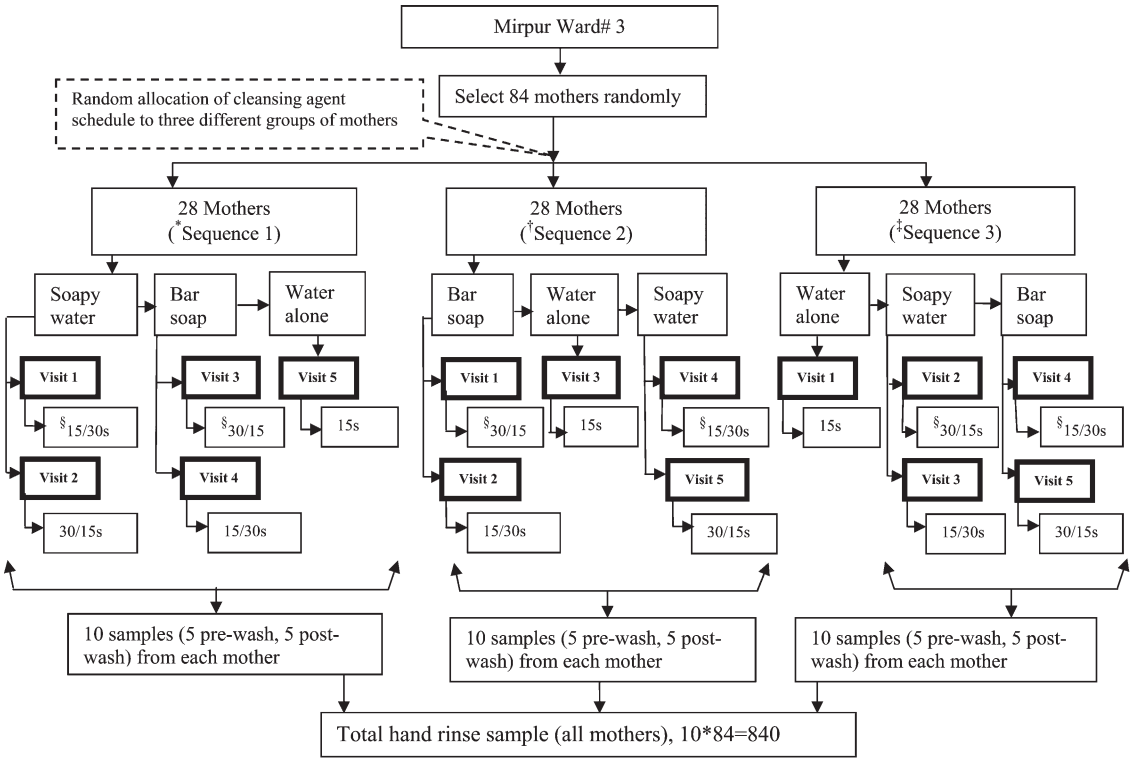
randomly selected 84 mothers from a participant list of the ongoing observational study. Using the same randomization technique, N.A. assigned 28 mothers each (from 84 selected mothers) to one of three different handwashing sequences using the three different cleansing agents (Figure 1). All selected mothers used three cleansing agents (soapy water [30 g powdered detergent in 1.5 L water], bar soap [Lux], and water alone; one agent per each visit).

Selection of hands and first scrubbing time. The investigator N.A. prepared 420 slips of paper, where hand selection (right versus left) and duration of scrubbing (15 or 30 seconds) were marked. The slips were placed within a envelope and shuffled to assure that no one can identify which envelope contains which hand and rubbing time. The fieldworker collected the required number of paper slips everyday in an envelope and as needed during that day, selected one slip at random to determine the hand with which each mother would start the assigned handwashing sequence; because the level of microbial hand contamination could differ in the left versus right hand,[8] we deemed it important to avoid bias that might result from systematically selecting either the right or left hand for hand rinse sampling. The fieldworker used the opposite hand of the mother on the subsequent visit to avoid the selection of the same hand. The same hand was not sampled two times, because the pre-wash hand rinse sampling method was similar to washing with water alone, and such pre-treatment would limit our ability to compare the efficacy of washing hands with soap/soapy water versus water alone.[21] The fieldworker also used the paper slips to randomly assign a scrubbing time of 15 or 30 seconds for both soapy water and bar soap and used only a 15 second scrubbing time for water alone. We used 15 and 30 seconds scrubbing times to ensure that the recommended scrubbing time was included.[22] A 15 second scrubbing time was close to the observed average hand scrubbing time of 12.5 seconds found in a study in urban Kamalapur, Dhaka[6] and the observed average hand scrubbing time of 14 seconds found in the United Kingdom.[23] Thirty seconds of scrubbing was the maximum scrubbing time observed after receiving the soap intervention in the Kamalapur study.[6] The fieldworker visited each mother five times to collect a total of 10 hand rinse samples from each mother (Figure 1).

During the first visit with the mother, fieldworkers described the study and after obtaining informed consent, administered a questionnaire on demographic characteristics and principle household water sources. During each of the five visits with the mother, fieldworkers collected information about recent hand hygiene practices, contact with feces from either their own defecation or cleaning a child who had defecated, and use of a cleansing agent for handwashing within the preceding 1 hour. The fieldworkers also observed hand cleanliness for both hands. We assigned hand cleanliness scores to three areas of the hand: fingernails, palms, and fingers. Fingernails included the fingernails, the skin under the fingernails, the skin directly surrounding the fingernails, and the cuticles. The palms included the inner surface of the hands not including the fingers, and the fingers included the base (proximal phalanges),



*Sequence 1: Soapy water, bar soap, water alone
†Sequence 2: Bar soap, water alone, soapy water
‡Sequence 3: Water alone, soapy water, bar soap
§For soapy water and bar soap groups, mothers were randomized to either 15sec or 30sec and then alternated scrubbing time in the subsequent visit.

FIGURE 1. Study design flowchart.

middle portion (middle phalanges), finger pads, and side of the fingers. Using pictorial cue cards, fieldworkers assessed the visible appearance of these three areas of both hands using a three-point scale: clean (observed part of the hand is clean as would appear after someone washes their hands or takes a bath), unclean (no dirt is visible on the hand, but part of the hand appears unclean), and dirty (visible dirt/mud/soil/ash or any other material).[21]

Hand rinse sample collection. After observing hand cleanliness, fieldworkers opened a sealed opaque envelope containing a paper slip indicating the random assignment of either the right or left hand for collecting a pre-wash hand rinse sample and either a 15 or 30 second scrubbing time with soapy water, bar soap, or water alone (Figure 1).

Before the prescribed handwashing, fieldworkers collected a pre-wash hand rinse sample by having the mother insert the selected hand into a sample collection bag (19 × 38 cm; Nasco Whirl-Pak, Fort Atkinson, WI) containing 200 mL sterile Ringer's solution and asking her to rub her fingers against her palm for 15 seconds. Then, fieldworkers massaged the inserted hand from the outside of the bag for an additional 15 seconds to ensure that all parts of the hand were fully immersed in the Ringer's solution. They closed the sample collection bag and immediately placed it into a cold box that was maintained at < 10°C with ice packs.[24]

After the pre-wash hand rinse sample was collected, fieldworkers showed a standard handwashing technique using a pictorial cue card (Supplemental Figure 1). They then requested that the mother wash both her hands with the hand cleansing agent as determined by the randomization. If the respondent was assigned to wash hands with soapy water, the mother wetted both of her hands with 10 mL water, and fieldworkers poured 20 mL soapy water solution onto the mother's hands. The mother scrubbed both her hands for the assigned duration (15 or 30 seconds), and then, fieldworkers poured 500 mL water over the mother's hands to rinse away the foam of the soapy water. Fieldworkers used a stopwatch to record the time of hand rinsing.[22] If the respondent was assigned to wash her hands with bar soap, she wetted her hands with 10 mL water and used a 100 g bar of soap to create foam. Fieldworkers then followed the same scrubbing and rinsing procedure as with soapy water. If the respondent was assigned to wash hands with water alone, fieldworkers poured water over the mother's hands while she scrubbed them for 15 seconds. The water used for hand wetting, scrubbing, and rinsing with soapy water, bar soap, and water alone was from the Dhaka municipal water supply collected from the household into a provided clean container.

After handwashing with the prescribed cleansing agent, fieldworkers collected a hand rinse sample from the hand that was not tested in the pre-wash hand rinse sample using similar techniques.

Municipal water testing. We tested samples from the Dhaka municipal water supply, the main source of water in the study participants' households, to determine the amount of indicator bacteria in the water that would be used for handwashing and other purposes. The field team used convenience sampling to select 10 households from five geographically different areas for source water testing. Fieldworkers collected a 100 mL water sample in a Whirl-Pak bag from the primary water source. If water was not available during the assigned visit from the primary water source, they collected a water sample from the household's stored water. Fieldworkers sealed the water sample bag and immediately placed it into a cold box.

Soapy water solution testing. The fieldworkers used convenience sampling to collect five water samples in five different 1.5 L plastic bottles from five different households (Water Supply and Sewerage Authority [WASA] tap or hand pump) and prepared the soapy water using the same recipe. Fieldworkers collected a 100 mL water sample in a Whirl-Pak bag from each soapy water bottle and immediately placed it into a cold box. The soapy water samples were tested to assess the load of thermotolerant coliforms in the WASA water after mixing the detergent powder.

Laboratory procedures. The International Center for Diarrheal Disease Research, Bangladesh (icddr,b) laboratory received the rinse water samples within 6 hours of collection and conducted a microbiological evaluation of each hand rinse sample using membrane filtration and the drop plate technique to detect thermotolerant coliforms and *C. perfringens*.[21] Thermotolerant coliforms are commonly used as indicators of fecal contamination in handwashing evaluations.[25–27] *C. perfringens* is a potential alternative biomarker of fecal contamination that persists in the environment for a longer period than other indicator organisms, such as *Escherichia coli* (a subset of fecal coliforms).[28] We chose to use thermotolerant coliforms to allow for comparison with other studies and selected *C. perfringens* to assess its use as a stable indicator of fecal contamination on hands.

For thermotolerant coliforms, 20 mL pre-wash and 50 mL post-wash hand rinse samples were filtered separately through 0.22-μm pore size membrane filters (Millipore Corp., Bedford, MA). Different volumes of hand rinse samples were filtered, because the pre-wash samples were more contaminated. The filter papers were then placed on plates of media prepared with mFC agar (Difco, MD). At the same time, 100 μL each hand rinse sample was taken directly from the sample bag using a micropipette (Labsystem, Australia) and inoculated onto the mFC agar plates using the drop plate technique.[29–31] The plates were then inoculated at 44°C for 18–24 hours. After incubation, characteristic blue colonies were counted as thermotolerant coliforms expressed as colony forming units (CFU) per hand (200 mL rinse solution). When the number of colonies was too numerous to count on the filter paper, the colonies enumerated by the drop plate technique were used to calculate CFU per hand.

To assess the concentration of *C. perfringens*, 20 mL pre-wash and 50 mL post-wash hand rinse samples were passed through 0.22 μm pore size membrane filters, placed onto plates containing modified *C. perfringens* medium (mCP; Oxoid, England), and incubated in an anaerobic jar at 44°C. After 24 hours, yellow colonies, characteristic of *C. perfringens*, were presumptively counted as *C. perfringens*. The yellow colonies were then exposed to 30% ammonium phosphate; the colonies that turned dark pink were confirmed as *C. perfringens*, and the count was expressed as CFU per hand.

Because the volume filtered differed for the pre- versus post-wash hand rinses, the lower limit of detection by membrane filtration was 10 CFU per hand for the pre-wash hand rinse samples and 4 CFU per hand for the post-wash hand rinse samples. The upper detection limit for all samples by drop plate technique was 100,000 CFU per hand. Levels of hand contamination with thermotolerant coliforms and

C. perfringens were $\log_{10}$-transformed to compare mean CFU per hand between groups.

From the municipal water and soapy water samples collected, 20 mL water was filtered through a 0.22 μm membrane filter, which was placed onto an mFC agar plate to test for thermotolerant coliforms. Then, the same procedure as for the hand rinse samples was followed.

SAMPLE SIZE CALCULATION AND STATISTICAL ANALYSIS

The concentration of bacteria was calculated as the number of CFU per hand and then converted to $\log_{10}$ counts for analysis. Non-inferiority involves selecting a meaningful difference between two groups that would indicate that they are different, and then calculating the sample size necessary to detect this difference. To achieve 80% power for detection of a non-inferiority difference margin of 0.50 $\log_{10}$ CFU between bar soap and soapy water, we calculated a required sample size of 84 mothers.[32] Therefore, we collected 420 paired hand rinse samples (five pre- and five post-treatment pairs for two different scrubbing times of 15 and 30 seconds) and analyzed them separately using the same regression model. After $\log_{10}$ transformation, we used paired *t* tests to evaluate the mean differences between the concentrations of indicator organisms in the pre-wash and post-wash hand rinses for each of the hand cleansing regimens. To estimate the difference between $\log_{10}$-transformed bacterial counts between pre- and post-wash (15 versus 30 seconds) and also the difference between handwashing agents, we used linear regression, where the dependent variable was the $\log_{10}$-transformed bacterial counts and independent variables were timing of hand rinse (pre- or post-wash), type of handwashing agent (soapy water or bar soap), and interaction between them. We also calculated the difference between the differences using the same regression model. To account for repeated measures (i.e., multiple hand rinse samples from the same mother), we used robust SEs to estimate 95% CIs. We used STATA 10 (StataCorp LP, College Station, TX) for analysis.

All participants provided written informed consent. The study protocol was reviewed and approved by the Ethics Review Committee of icddr,b.

RESULTS

Household characteristics. We enrolled 84 participants into the study. The characteristics of the randomly assigned groups of mother were comparable (Supplemental Table 1). The mean age of the enrolled mothers was 26 years (SD = 0.5, range = 18–38 years). They completed a mean 3 years of formal education and lived in households with a mean of five members. Among 84 participating households, 82 (98%) households had water supplied by the Dhaka municipality. All 10 source water samples taken were contaminated with thermotolerant coliforms (mean $\log_{10}$ = 3.2 CFU/100 mL, range = 2.3–5.2 $\log_{10}$ CFU/100 mL), but 5 soapy water solution samples were free from thermotolerant coliforms (0 CFU/100 mL).

Reported hand hygiene behavior. Reported hand hygiene behavior within the last 1 hour and the level of visible hand contamination (dirty, unclean, and clean) before washing were similar across all mothers, regardless of the cleansing agent used. One-third of the mothers reported that at least one hand came into contact with feces after they either defecated or cleaned a child who had defecated; about one-third reported not washing their hands within the last 1 hour. More than 90% of mothers reported washing their hands with soap after defecation and cleaning a child's anus, but only 18% of mothers reported washing hands with soap before eating and/or feeding a child. On observation, 75% of study participant's hands were visibly clean, with the palms of both hands being visibly cleaner than the fingernails (Table 1).

Fieldworkers collected 840 (100%) hand rinse samples (420 pre-wash [210 right and 210 left hands] and 420 post-wash [210 right and 210 left hands] samples). More than 90% of the pre-wash hand rinse samples were contaminated with thermotolerant coliforms, and more than 70% were contaminated with *C. perfringens*; the mean $\log_{10}$ concentration was similar across groups for both organisms (Table 2). Right hands (N = 210) were more contaminated with thermotolerant coliforms in the pre-wash samples (mean $\log_{10}$ difference of right hands minus left hands = 0.36 CFU, 95% CI = 0.10–0.61, P = 0.0058), but in the post-wash samples, right and left hands (N = 210) were equally contaminated (mean $\log_{10}$ difference of right hands minus left hands = –0.08 CFU, 95% CI = –0.29–0.14, P = 0.48). The concentrations of *C. perfringens* in both pre-wash and post-wash samples were equal for both right and left hands.

Microbiological effectiveness. Scrubbing hands for 15 seconds with soapy water reduced thermotolerant coliforms to a similar degree as observed with washing with bar soap (mean $\log_{10}$ reduction = 0.7 CFU per hand, 95% CI = 0.44–0.92, P < 0.001 for soapy water and mean $\log_{10}$ reduction = 0.6 CFU per hand, 95% CI = 0.24–0.95, P = 0.001 for bar soap). There were also significant reductions in the concentrations of *C. perfringens* (mean $\log_{10}$ reduction = 0.8 CFU per hand, 95% CI = 0.65–1.0, P < 0.001 for soapy water and mean $\log_{10}$ reduction = 0.8 CFU per hand, 95% CI= 0.64–1.0, P < 0.001 for bar soap) after scrubbing hands with soapy water and bar soap for 15 seconds. We calculated that it took an average of 12 seconds (range = 10–15 seconds, SD = 0.07) to remove the foam produced during scrubbing for both the soapy water and bar soap. Washing hands with water alone for 15 seconds also reduced the load of thermotolerant coliforms on hands (mean log reduction = 0.3 CFU per hand, 95% CI = 0.004–0.57, P = 0.047) but to a lesser degree than washing with soapy water or bar soap. The reduction of thermotolerant coliforms was significantly higher after scrubbing with soapy water compared with scrubbing with water alone ($\log_{10}$ mean difference of thermotolerant coliforms between soapy water and water alone = –0.4 CFU per hand, 95% CI = –0.76–0.02, P = 0.038) but not significantly higher in reducing *C. perfringens* ($\log_{10}$ mean difference of *C. perfringens* between soapy water and water alone = –0.15 CFU per hand, 95% CI = –0.40–0.09, P = 0.22). Increasing the scrubbing time from 15 to 30 seconds with either soapy water or bar soap did not significantly increase the microbiological effectiveness of removing fecal indicator organisms (Table 2).

Adjustment for visible hand contamination (visibly clean versus visibly dirty hands) before handwashing did not significantly change the effectiveness of the handwashing agents in removing fecal indicator organisms (Table 3).

DISCUSSION

Soapy water has been developed and promoted as a low-cost alternative to bar soap for handwashing in Kenya,[14] Bangladesh,[16–18] and Peru.[15] In a study conducted by icddr, b, a family of five members used a 1.5 L mixture of soapy water in 8–10 days on average for handwashing near the latrine and 14–15 days near the kitchen (Alam FN, personal communication). This time is similar to the mean use time for a bar of soap (13 days).[33] In our study, soapy water performed as well as bar soap at reducing thermotolerant coliforms and *C. perfringens* on hands in a low-income urban community with a highly contaminated water supply. We tested for *C. perfringens*, because it would indicate that soapy

TABLE 1

Reported hand hygiene behavior within the previous 1 hour and appearance of mother's hands immediately before sample collection in Mirpur, Dhaka in 2011

Reported and observed behavior	Soapy water ($N = 168$)* n (%)	Bar soap ($N = 168$)* n (%)	Water alone ($N = 84$) n (%)	Total ($N = 420$) n (%)
Reported hand(s) contact with feces (self or child's) within last 1 hour	61 (36)	51 (30)	24 (29)	136 (32)
Reported handwashing within 1 hour preceding pre-wash hand rinse sampling	129 (77)	128 (76)	67 (80)	324 (77)
Hand(s) reported washed within the previous 1 hour				
Right hand	5 (4)	9 (7)	1 (1.5)	15 (4)
Left hand	3 (2)	5 (4)	1 (1.5)	9 (2)
Both hands	121 (94)	114 (89)	65 (97)	300 (71)
Handwashing agent used within the last 1 hour (multiple responses allowed)				
After defecation				
Any soap and water (soap, soapy water, or detergent)	27 (96)	19 (83)	13 (100)	59 (92)
Water only	1 (4)	3 (13)	0	4 (6)
Other	0	1 (4)	0	1 (2)
After toileting				
Any soap and water	6 (14)	1 (4)	3 (19)	10 (12)
Water only	37 (86)	24 (96)	13 (81)	74 (88)
After cleaning child's anus				
Any soap and water	36 (86)	35 (92)	17 (100)	88 (91)
Water only	6 (14)	1 (3)	0	7 (7)
Other	0	2 (5)	0	2 (2)
During bathing				
Any soap and water	14 (88)	9 (90)	4 (100)	27 (90)
Water only	2 (12)	1 (10)	0	3 (10)
Before eating and feeding				
Any soap and water	7 (13)	11 (24)	3 (18)	21 (18)
Water only	46 (87)	34 (76)	14 (82)	94 (82)
After household or kitchen works				
Any soap and water	52 (58)	38 (45)	25 (57)	115 (53)
Water only	37 (42)	46 (55)	19 (43)	102 (47)
How many times hand(s) was washed within the previous 1 hour				
No handwashing	39 (23)	40 (24)	17 (20)	96 (23)
One time	36 (21)	51 (30)	31 (37)	118 (28)
Two times	42 (25)	42 (25)	18 (22)	102 (24)
More than two times	51 (31)	35 (21)	18 (21)	104 (25)
Appearance of right hand: fingernails				
Dirty†	16 (10)	5 (3)	8 (10)	29 (7)
Unclean‡	63 (38)	69 (41)	25 (30)	157 (37)
Clean§	89 (53)	94 (56)	51 (61)	234 (56)
Appearance of right hand: palms				
Dirty	4 (2)	1 (0.6)	1 (1)	6 (1)
Unclean	33 (20)	14 (8)	7 (8)	54 (13)
Clean	131 (78)	153 (91)	76 (91)	360 (86)
Appearance of right hand: fingers				
Dirty	5 (3)	1 (0.6)	1 (1)	7 (2)
Unclean	32 (19)	22 (13)	9 (11)	63 (15)
Clean	131 (78)	145 (86)	74 (88)	350 (83)
Appearance of left hand: fingernails				
Dirty	16 (10)	9 (5)	13 (15)	38 (9)
Unclean	78 (46)	72 (43)	27 (32)	177 (42)
Clean	74 (44)	87 (52)	44 (52)	205 (49)
Appearance of left hand: palms				
Dirty	5 (3)	1 (0.6)	2 (2)	8 (2)
Unclean	34 (20)	15 (9)	9 (11)	58 (14)
Clean	129 (77)	152 (90)	73 (87)	354 (84)
Appearance of left hand: fingers				
Dirty	4 (2)	1 (0.6)	2 (2)	7 (2)
Unclean	34 (20)	27 (16)	10 (12)	71 (17)
Clean	130 (77)	140 (83)	72 (86)	342 (81)

*Data collected at two different visits: 15- and 30-second scrubbing times; $N = (84 \times 2)$.
†Dirty: Visible dirt/mud/soil/ash or any other material.
‡Unclean: No dirt is visible on the hand, but part of the hand appears unclean.
§Clean: Observed part of the hand is clean (as it would appear after someone washes their hands or takes a bath).

TABLE 2

Comparison of mean log concentration of thermotolerant coliforms and *C. perfringens* per hand of water alone, bar soap, and soapy water within group and between groups for both 15 and 30 second scrubbing among mothers in Mirpur, Dhaka in 2011

	Mean log CFU per hand (95% CI)											
	Soapy water group			Bar soap group			Water alone group			Difference in differences between groups (*P* value)		
Scrubbing time and indicator organisms	Pre-wash (*N* = 168)	Post-wash (*N* = 168)	Difference within group	Pre-wash (*N* = 168)	Post-wash (*N* = 168)	Difference within group	Pre-wash (*N* = 84)	Post-wash (*N* = 84)	Difference within group	Bar soap minus soapy water	Water alone minus soapy water	Water alone minus bar soap
15 seconds of scrubbing												
Thermotolerant *coliforms*	2.5	1.8	0.7 (0.44–0.92)	2.7	2.1	0.6 (0.24–0.95)	2.3	2.0	0.3 (0.004–0.57)	–0.09 (–0.51–0.34)	–0.4 (–0.76 to –0.02)	–0.3 (–0.76–0.14)
C. perfringens	1.2	0.4	0.8 (0.65–1.0)	1.2	0.4	0.8 (0.64–1.0)	1.2	0.6	0.7 (0.49–0.85)	–0.004 (–0.27–0.26)	–0.15 (–0.40–0.09)	–0.15 (–0.41–0.11)
30 seconds of scrubbing												
Thermotolerant coliforms	2.7	2.0	0.7 (0.46–0.96)	2.7	2.2	0.5 (0.24–0.75)	NA	NA	NA	–0.22 (–0.57–0.14)	NA	NA
C. perfringens	1.0	0.3	0.7 (0.52–0.91)	1.2	0.3	0.9 (0.74–1.1)	NA	NA	NA	0.21 (–0.05–0.46)	NA	NA

NA = not applicable.

TABLE 3

Mean log concentration of thermotolerant coliforms and *C. perfringens* before and after washing both hands with soapy water, bar soap, and water alone based on observations of visibly dirty and visibly clean hands in Mirpur, Dhaka in 2011

	Mean log CFU per hand (95% CI)								
	Soapy water			Bar soap			Water alone		
Indicator organisms in hand rinse samples	Visibly dirty hands* (*N* = 140)	Visibly clean hands† (*N* = 196)	Difference	Visibly dirty hands* (*N* = 168)	Visibly clean hands† (*N* = 168)	Difference	Visibly dirty hands* (*N* = 74)	Visibly clean hands† (*N* = 94)	Difference
Thermotolerant coliforms									
Pre-wash	2.6	2.5		2.7	2.7		2.4	2.2	
Post-wash	2.0	1.7		2.2	2.1		2.2	1.9	
Differences	–0.6	–0.8	0.2	–0.5	–0.5	–0.006	–0.25	–0.3	–0.08
	(–0.87 to –0.37)	(–1.1 to –0.54)	(–0.18–0.55)‡	(–0.87 to –0.22)	(–0.83 to –0.25)	(–0.42–0.4)‡	(–0.64–0.14)	(–0.76–0.09)	(–0.49–0.66)‡
C. perfringens									
Pre-wash	1.1	1.2		1.2	1.3		1.2	1.3	
Post-wash	0.4	0.4		0.4	0.4		0.7	0.4	
Differences	–0.7	–0.8	0.07	–0.8	–0.9	–0.1	–0.4	–1.0	0.5
	(–0.92 to –0.56)	(–1.0 to –0.6)	(–0.19–0.33)‡	(–0.98 to –0.63)	(–1.1 to –0.76)	(–0.13–0.4)‡	(–0.67 to –0.21)	(–1.2 to –0.72)	(0.19–0.87)‡

* Visible dirt/mud/soil/ash or any other material on any part of both hands and no dirt visible on the hand, but in general, part of the hand appears unclean.
† All parts of both hands were clean as they would appear after someone washes hands or takes a bath.
‡ Difference between mean difference of before and after washing hands and visibly dirty and clean hands.

water also works well on hardier organisms. Handwashing with water alone also worked well in reducing *C. perfringens*, indicating that the removal of these organisms may be mostly a physical process from the friction of rubbing hands together.

In Bangladesh, the average price of a common bar soap (Lux) is US$0.35, whereas a mixture of soapy water costs US $0.03. The 1.5 L plastic bottle (e.g., mineral water bottle or soft drink/juice bottle), which costs US$0.06, could be reused as long as it continues to hold water. Because we estimate that soapy water lasts the same duration as soap (three bar soaps or three soapy water bottles required per month per household), its use would cost only US$0.15 (three sachets of detergent cost US$0.09 + one plastic bottle costs US$0.06) in the first 1 month and US$0.09 for each subsequent 1 month. This cost provides a savings of US$0.90 in the first 1 month and US$0.96 for each subsequent 1 month compared with bar soap. Households may find cost saving an added incentive for adopting soapy water or having multiple bottles of soapy water to use at different sites within the home, which may facilitate handwashing at times of possible pathogen transmission to or from hands.[11,34]

Our microbial evaluation showed that handwashing with water alone also reduced the level of both thermotolerant coliforms and *C. perfringens* on hands, although the reduction was significantly lower than for handwashing with soapy water ($\log_{10}$ mean difference of thermotolerant coliforms between soapy water and water alone = −0.4 CFU per hand, 95% CI = −0.76 to −0.02, $P = 0.038$). A recent laboratory-based study in the United Kingdom similarly found that the presence of fecal bacteria was reduced by 23% by handwashing using water alone.[23] A community-based observational study in Bangladesh reported that children who lived in households where food preparers briefly washed their hands with water alone experienced significantly less diarrhea than children living in households where food preparers did not wash their hands at all.[4] Other evaluations suggest that washing hands with water alone can reduce the amount of bacteria[8,28,35,36] but not to the same extent as bar soap. Conversely, Hoque and Briend,[7] from a small community-based handwashing study in Dhaka, suggested that washing hands with soap, mud, or ash significantly removed fecal coliforms from hands, whereas washing with water alone did not. However, their study measured only presence or absence of fecal coliforms. Our study enrolled nearly five times as many participants and was designed to have sufficient power to detect differences in microbial concentrations after washing hands with soapy water, bar soap, and water alone.

Mothers in our study washed their hands with contaminated water ($\log_{10}$ mean of thermotolerant coliforms/100 mL water = 3.2 CFU, range = 2.3–5.2). We did not evaluate handwashing with contaminated water against handwashing with uncontaminated water, but a community-based study in Pakistan found that difference in the level of hand contamination did not differ significantly for households provided bacteria-free chlorinated water for handwashing versus households that used heavily contaminated municipal water for handwashing.[35] These findings contrast with a study in Uttarkhan, Bangladesh that suggested the hands of women who washed their hands with highly contaminated pond water (geometric mean of the count of fecal coliforms = 17,330/100 mL) were significantly more contaminated compared with the hands of women who washed their hands with less contaminated tube well water (geometric mean of the count of fecal coliforms = 32/100 mL).[25] Perhaps handwashing with either soapy water or bar soap in our study would have resulted in additional reduction of hand contamination if the hands were washed using uncontaminated water.[35,37] Although we only tested a small sample of municipal water, our data suggest that even using moderately contaminated water for handwashing can still reduce fecal contamination from hands. More generally, our study supports that handwashing should be encouraged even when available water is bacterially contaminated.

Effective handwashing to reduce bacteria from hands depends on several factors: duration of handwashing, type and volume of cleansing agent, and quality of water.[21,25,34] A US laboratory-based study, focusing on duration and type of cleansing agent, found that antimicrobial soap reduced *Shigella flexneri* significantly more at 30 seconds than 15 seconds, but plain soap did not.[21] A US hospital-based study found that washing hands with plain soap and water for 15 seconds reduced bacterial counts on the skin by 0.6–1.1 $\log_{10}$, whereas washing hands with plain soap and water for 30 seconds reduced counts by 1.8–2.8 $\log_{10}$.[38] These differences in hand contamination might be because of different study contexts. A laboratory or hospital setting in a high-income country is likely to be far less contaminated and have cleaner water than the study area and municipal water in Bangladesh. Scrubbing hands with soapy water, bar soap, or water alone for longer than 15 seconds may be unlikely to remove additional bacteria when the water itself is contaminated.

Several studies used stopwatches to measure handwashing duration[6,23,39–41] but did not explore the handwashing process by separating scrubbing times and rinsing times. A range of timings of both scrubbing and rinsing hands has been recommended by previous studies.[42–47] We recommend a 15 second scrubbing time, because it was as effective as 30 seconds in removing indicator organisms and is closer to the duration of handwashing typically carried out by persons in Bangladesh.[6] This time is below the 20 second scrubbing time recommended by the Centers for Disease Control and Prevention (CDC)[42] and United Nations Children's Fund (UNICEF)[47] and may improve adherence to handwashing recommendations.

Visible appearance of hand cleanliness was not associated with the level of hand contamination or the efficacy of soapy water or bar soap in removing thermotolerant coliforms and *C. perfringens* from hands. A similar result was found in a handwashing intervention with hand sanitizer and liquid soap in Dar es Salam, Tanzania, which suggested that the efficacy of handwashing with soap was not associated with visible dirt on the hands.[25]

There are important limitations to this study. First, we used supervised handwashing regimens with pre-specified hand scrubbing times and hand lathering motions.[9] Although this prescriptive approach strengthens the internal validity of our study, the reduction of microbial load by washing hands with soapy water or bar soap may be different if community members were to wash their hands for a shorter time or did not use the comprehensive scrubbing motions that we promoted. Additional research should explore the effectiveness of soapy water when not directly supervising community members' handwashing. Second, we used 500 mL municipal water during the handwashing procedure to rinse away foam. In settings where water is less plentiful, results may be different.

Additional research is necessary to explore the effectiveness of soapy water in settings where water is scarce. Third, viruses and specific pathogens that can also cause diarrhea were not measured in this study, and the efficacy of soapy water on removal of these pathogens could differ.

Soapy water may be promoted as a low-cost but similarly effective alternative to bar soap for cleaning hands to reduce bacterial contamination and potentially reduce enteric disease transmission. This low-cost and readily available alternative may increase handwashing frequency, which ultimately may reduce disease burden globally. This intervention may be particularly appropriate for low-income communities, where there are concerns about the cost of bar soap. Additional studies could explore the efficacy of soapy water in removing viruses and assess the effect of handwashing with soapy water on health outcomes, such as diarrhea and respiratory diseases.

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