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Microbiologic effectiveness of hand washing with soap in an urban squatter settlement, Karachi, Pakistan

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SUMMARY

We conducted a study in a squatter settlement in Karachi, Pakistan where residents report commonly washing their hands to determine if providing soap, encouraging hand washing, and improving wash-water quality would improve hand cleanliness. We allocated interventions to 75 mothers and collected hand-rinse samples on unannounced visits. In the final model compared with mothers who received no hand-washing intervention, mothers who received soap would be expected to have 65% fewer thermotolerant coliform bacteria on their hands (95% CI 40%, 79%) and mothers who received soap, a safe water storage vessel, hypochlorite for water treatment, and instructions to wash their hands with soap and chlorinated water would be expected to have 74% fewer (95% CI 57%, 84%). The difference between those who received soap alone, and those who received soap plus the safe water vessel was not significant ($P = 0{\cdot}26$). Providing soap and promoting hand washing measurably improved mothers' hand cleanliness even when used with contaminated water.

INTRODUCTION

The World health organization estimates that each year over 2 million children die of diarrhoea, almost all of them in developing countries. [1]. Promoting hand washing is an affordable intervention. Intensive community-based interventions to promote hand washing in communities with a high diarrhoeal incidence, have decreased diarrhoeal incidence by 26–62% [2–5].

However, there are barriers to widespread effective hand washing. In settings where most diarrhoeal deaths occur, water is contaminated with human faeces. Field studies suggest that poor microbiologic quality of water decreases hand washing effectiveness [6]. Achieving effective hand washing behaviour is also difficult. Even in Islamic countries where hand washing is a religious practice [7], it is often practised inconsistently or with techniques that do not ensure

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microbiologic cleanliness [6, 8]. Evaluating hand washing promotion efforts is difficult, because in settings where hand washing is part of the cultural expectation, there is a substantial difference between reported and actual hand washing behaviour [8, 9].

In Karachi, Pakistan, over 4 million Muslim residents live in squatter settlements. Available water in these settlements is heavily contaminated with faecal bacteria; there is no regular system of garbage collection and removal, and sewage commonly drains through open ditches [10]. Population based verbal autopsy studies in five of these squatter communities measured an under 5-year-old mortality rate of 100 deaths per 1000 live births; diarrhoeal diseases were the primary cause of 39% of these deaths [11].

We conducted a hand washing promotion study in Karachi to answer two questions. First, in a highly contaminated environment with multiple opportunities for recontamination and where hand washing was already commonly reported, would community-based efforts to promote hand washing improve hand cleanliness? Second, would the additional expense and effort of providing cleaner, chlorinated water for hand washing result in microbiologically cleaner hands?

MATERIAL AND METHODS

Setting

We conducted the study in Manzoor Colony, a typical multiethnic squatter settlement in central Karachi where a local non-government organization, Health Oriented Preventive Education (HOPE), manages a community-based primary health care programme. HOPE was trusted by the community and interested in active collaboration on the project. Only households where children under age 5 years were living were eligible for the study. We administered a pre-intervention survey to assess socioeconomic status, water availability and water use habits among candidate households in all three intervention sectors.

Interventions

There were two physical interventions. First, we provided Safeguard®† soap to selected participants. Safeguard® contains 1·2% triclocarban which has specific antibacterial activity against *Staphylococcus* sp., but is not active against thermotolerant coliforms or other Gram negative bacteria [12]. Study personnel gave white bars of Safeguard® to mothers with children under age 5 years and encouraged the mothers to use this soap exclusively when washing their own hands. We provided beige coloured bars of Safeguard® for all other family members in the participating household. Study personnel returned to the households twice weekly to replenish soap supplies. They provided a maximum of one bar of soap of each colour at each visit. The bars were distributed unwrapped to discourage households from selling them.

Second, water storage vessels and chlorine bleach were provided to selected households. The 20-litre vessels had a narrow mouth specifically designed to prevent entry of hands into the vessel, and a high quality tap ([Catalog No. 420-812; Tolco, Inc., Toledo, OH]) [13]. Households were given dilute household bleach (sodium hypochlorite) in individual 10-ml bottles. The concentration was titrated so that when added to the vessel, it consistently produced a free chlorine concentration > 1·0 mg/l in Manzoor colony water in multiple samples before the study began.

This evaluation of hand washing was part of a project that also evaluated the effect of using the water storage vessel with home chlorination on drinking water quality [14]. There were three intervention groups of 25 households each. Group 1 received soap and instructions to wash their hands using available water. Group 2 received both soap and the water storage vessel with bleach. They were instructed to chlorinate the water and use it for both drinking and hand washing. Group 3 received the water storage vessel and bleach for drinking water only. They were given no soap and no instructions for hand washing. The three groups were in geographically separate sectors of Manzoor colony, so that community-based messages encouraging behaviour change were distinct for each intervention.

We developed slide shows, video tapes and written pamphlets to illustrate the risks of hand contamination and specific instructions on how to wash hands thoroughly and how to use the vessel and dilute hypochlorite to purify and store water. Field workers instructed mothers in households receiving soap to wash their hands routinely, but especially after defaecation and before preparing food. Group meetings and materials were supplemented by home meetings with community health workers who demonstrated thorough hand washing technique and

† Inclusion of trade names is for identification only and does not imply endorsement by CDC or the Department of Health and Human Services.

encouraged mothers to wash their hands frequently. During the intervention, community health workers returned to enrolled households on average twice weekly to encourage their ongoing participation.

Hand rinse cultures

We prepared sterile 1-litre sample collection bags (Whirl-Pack® Nasco, Fort Atkinson, WI) containing 125 ml of 0·1 % peptone broth. At baseline, and 1, 3, 6 and 10 weeks later, the study team approached each household in an unannounced visit. They asked the mother to insert one hand at a time into the sterile collection bag and rotate each hand for 5 sec to rinse it thoroughly in the peptone broth. The broth samples were placed in a cooler on ice, and transported to the Aga Khan University Hospital Laboratory for analysis within 4 h of collection.

Laboratory personnel initially prepared broth at dilutions 1:1, 1:10 and 1:100 with 0·01 M sterile phosphate buffered saline. Because the thermotolerant coliforms on many of the initial baseline samples were too numerous to count, a 1:1000 dilution was later substituted for the 1:1 dilution. Laboratory personnel filtered 50 ml of each dilution through a sterile 0·45-µm paper filter. For thermotolerant coliform concentration, they transferred each filter to a 60-mm petri plate containing EMB agar and incubated the plates at 44 °C for 24 h.

Baseline drinking water cultures

We collected baseline water samples in 120-ml sterile plastic containers, placed them in a cooler on ice, and transported them to the Aga Khan University Hospital Laboratory for analysis within 4 h of collection. For microbiologic evaluation, we tested water samples at three concentrations: undiluted, diluted 1:10, and diluted 1:100 with 0·01 M sterile phosphate buffered saline. We filtered 50 ml of each dilution through a sterile 0·45-µm paper filter and transferred each filter to a 90-mm petri plate containing EMB agar. We incubated the plates at 44 °C for 24 h, then examined them for thermotolerant coliform and *Escherichia coli* colony counts.

Statistical analysis

The countable range of colonies was 10 to 100. If the number of colonies in only one plate was within the countable range, this count was used to estimate bacterial density. If two or more plates had colonies within the countable range, we estimated bacterial density by calculating the arithmetic mean of the counts of these plates. If one or more filters had colonies too numerous to count and the more dilute filters had colonies below the countable range, the bacterial density was estimated to be at the maximum countable concentration of the most dilute filter that had colonies too numerous to count. If all the filters had colonies too numerous to count, the bacterial density was estimated to be twice the upper limit of the countable range.

We calculated that 18 households per intervention would be sufficient assuming that 85 % of households would have contaminated water at baseline and that the intervention would reduce that to 30 %, with 80 % power and 95 % confidence, We increased the number to 25 per group to permit a greater diversity in experiences with the intervention, and to allow for dropout.

To assess the role of chance in explaining differences between groups, we used the t-test when comparing normally distributed means. When comparing prevalences we used the χ^2-test, or 2-sided Fisher's exact test when the expected cell size was < 5. Because the estimated concentrations of thermotolerant coliforms varied exponentially and were not normally distributed, we calculated geometric mean concentrations and used the Wilcoxon rank sum test to assess whether differences in the distribution of concentrations between groups were likely due to chance. We used simple linear regression of the log transformed levels of hand contamination versus week of sample collection to assess time trends. We used a general linear model to adjust for baseline differences between the intervention groups and to evaluate if the differences in geometric mean concentration of thermotolerant coliforms between groups were greater than would be expected by chance. We extended the general linear model through standard covariate structures, including unstructured, compound symmetry and autoregressive parameters to account for repeated measures. We used Epi Info [15] for data management and univariate statistical calculations and SAS [16] for the general linear modeling.

Ethics

As part of the process of informed consent, the field team explained the purpose of the study to each prospective participant. They emphasized the voluntary nature of the project and the opportunity to withdraw at any time from the study. The study protocol was reviewed and approved by the Aga Khan University Human Subjects Committee.

RESULTS

Of the 75 households enrolled in the study, 5 withdrew before their first post-intervention samples were collected. An additional 12 households were eliminated from the analysis because their baseline hand rinse samples were assessed at a maximum dilution of 1:100 and the result was too numerous to count and therefore not comparable to subsequent samples collected from that and from other households, which were analysed at 1:1000 dilution. So, 58 households – 17 which received only soap; 21 which received soap, a water vessel and bleach; and 20 which received no soap – were included in this analysis. The study team collected 231 of the 232 scheduled follow-up samples from the 58 households. Compared with the 58 households included in the analysis, the 17 that were excluded from the analysis had a similar prevalence of maternal literacy (57% among included *vs.* 65% among excluded, $P = 0{\cdot}76$), were as likely to have a constant municipal water connection (36% *vs.* 41%, $P = 0{\cdot}71$), had drinking water that was similarly contaminated at baseline (geometric mean concentration of thermotolerant coliforms 9885 *vs.* 9931 colony forming units (c.f.u.)/ml, $P = 0{\cdot}98$), and spent a similar amount on soap each month (114 *vs.* 127 Pakistani rupees, $P = 0{\cdot}42$).

The three groups of houses included in the analysis were of similar size and had similar water storage, sanitation, and refrigeration facilities (Table 1). At baseline, drinking water was heavily contaminated in all three groups, with an overall geometric mean of 9885 c.f.u. of thermotolerant coliforms per 100 ml. A higher proportion of mothers in the group receiving only soap were literate (81%) than in the group who received soap and the vessel (43%) or the group who received no soap (55%). Municipally supplied water typically runs 2–3 h per day in these neighbourhoods. When the water starts to run, residents turned on electric pumps to draw the maximum amount of water through rubber hoses connected to community water taps into their household storage tanks. Eighty-two percent of households receiving only soap had their own pump with a rubber hose always connected to a tap so that when water ran they could collect it. Only 10% of households that received soap and the vessel and 25% of households that received no soap had such a direct municipal water connection. Households without their own tap shared a municipal tap with two or three neighbouring households, and so used their own pump to collect water on alternate days. Households that received no soap, reported spending more money per month on soap (3·17 US$) than either households that received only soap (2·62 US$) or those that received soap, bleach, and the vessel (1·84 US$) (Table 1).

During the intervention, mothers who received only soap used an average 0·64 bars of white soap per week, compared with 0·66 bars among mothers who received both soap and the water vessel ($P = 0{\cdot}32$).

In all three groups mothers' hands were heavily contaminated. At baseline the 125 ml of hand rinse solution contained a geometric mean of 17140 thermotolerant coliforms. Mothers in the group receiving no soap had the most heavily contaminated hands at baseline (25410 thermotolerant coliforms), mothers receiving only soap (16256) and mothers receiving soap and a water vessel (12331). With the large variance among samples, the differences in baseline hand contamination were not statistically significant ($P = 0{\cdot}44$).

During follow-up the mothers' hand rinse samples from the vessel only group contained a geometric mean 30479 thermotolerant organisms, compared to 8492 organisms ($P < 0{\cdot}001$) among the supplemental soap group and 4819 among the supplemental soap and vessel group ($P < 0{\cdot}001$).

The levels of hand contamination also varied by week. At week 1 the geometric mean hand contamination was 11143 thermotolerant coliforms. It peaked at 20512 in week 3, and then decreased to 7228 in week 6 and was 7907 in week 10. Even with the intervention in progress the mean level of hand contamination noted in week 3 was higher than the pre-intervention level. Week 3 coincided with preparations for the Eid holiday. Mothers in these communities have numerous extra responsibilities to prepare for these festivities.

Baseline difference in hand contamination were controlled by subtracting each mother's pre-intervention sample from their four subsequent evaluations. The geometric mean number of thermotolerant coliforms on the hands' of mothers who received no supplemental soap increased by an average of 16% ($P = 0{\cdot}55$), but decreased 42% among mothers who received only soap ($P = 0{\cdot}08$), and 63% among mothers who received soap, a water vessel, and bleach ($P = 0{\cdot}001$).

Table 1. *Baseline characteristics among the three intervention groups, Manzoor Colony, Karachi, 1997*

Characteristic	Soap only ($n = 17$)	Soap + vessel ($n = 21$)	No soap ($n = 20$)	*P*-value*
No. of persons in the household	6·8	7·9	8·6	0·14
Duration of residence (years)	21	19	23	0·55
No. of children under age 5 years in the house	1·9	1·9	2·2	0·58
Literacy among mothers (%)	81	43	55	0·06
Median household income range	3001–4000 Rs	3001–4000 Rs	3001–4000 Rs	
Municipal water connection to house (%)	82	10	25	< 0·001
Covered concrete tank for major household water storage (%)	88	86	85	0·96
Refrigerator in house (%)	47	48	50	0·98
Boil drinking water for children (at least sometimes) (%)	50	28	35	0·34
Toilet without flush tank (%)	100	95	95	0·65
Geometric mean concentration of thermotolerant coliforms in drinking water (c.f.u./100 ml)	10864	8241	10568	0·26
Mean bars of soap used per month	9	7	11	0·10
Mean amount spent on soap per month (US$)	2·62	1·84	3·17	0·005

* Based on analysis of variance for comparison of continuous variables with equivalent variances, Kruskal–Wallis for comparison of continuous variables with different variances, and χ^2 for categorical variables.

Table 2. *Thermotolerant coliforms recovered from mothers' hands at baseline and post intervention, Manzoor Colony, 1997–8*

	Thermotolerant coliforms in c.f.u./125 ml (*P*-value*)				
Group	Baseline	Week 1	Week 3	Week 6	Week 10
Vessel only ($n = 20$)	25410	26002	79615	16218	25351
Soap only ($n = 17$)	16256	6427	15276	5047	10280
	(0·472)	(0·014)	(0·012)	(0·017)	(0·143)
Soap + vessel ($n = 21$)	12,331	7621	7129	4375	2254
	(0·166)	(< 0·001)	(0·005)	(0·154)	(0·001)
P-value soap *vs.* soap + vessel	0·444	0·275	0·385	0·658	0·042

* *P*-value compares the log concentration in the intervention groups to the no soap (vessel only) group based on the Kruskal–Wallis test.

To control for the effect of time, mothers from the group who received no soap but whose sample was collected during the same week were used as the comparison group. By this analysis both the mothers who received soap and the mothers who received soap and the water vessel had significantly fewer thermotolerant coliforms on their hands at 3 of the 4 follow-up evaluations (Table 2). The level of hand contamination was similar between the mothers who received only soap and those who received soap and the water vessel. Only on the fourth and final evaluation, at week 10, did the mothers who received soap and the hypocholorite and vessel have significantly fewer thermotolerant organisms on their hands than those who received soap alone (Table 2).

Among mothers who received the soap and vessel their geometric mean level of hand contamination was lower at each evaluation decreasing from 7621 at week 1 to 2254 at week 10. With the large variance in the data, this log linear decline was no greater than would be expected by chance ($R = -0·20$, 95% CI $-0·42$, 0·50).

The general linear model controlled for both the differences in baseline contamination among groups and the different levels of contamination at different times. It used the log of the number of thermotolerant coliforms on the hands of mothers from the unannounced follow up visits as the outcome, and baseline hand contamination, baseline water contamination, maternal literacy, presence of piped water in the household, amount of money spent on soap, the week of sample collection, and the intervention group assignment as predictor variables, The model generated parameters which estimated how much an incremental change in each of the predictive factors would be expected to affect the log of the number of thermotolerant coliforms. One household was excluded from the multivariate analysis because there was no information on maternal literacy. Four of the remaining 227 observations were excluded as outliers. In the final model, the only variables that were significantly associated with the level of hand contamination at the unannounced follow-up visits were the level of hand contamination at baseline, the week of sample collection, and the intervention group. There were no significant interactions. After adjustment, compared with persons who received no soap, persons who received soap would be expected to have 65% fewer thermotolerant coliforms on their hands (95% CI 40%, 79%), and persons who received the soap and vessel 74% fewer (95% CI = 57%, 84%). The difference in hand contamination between those who received soap alone, and those who received soap plus the clean water vessel may have been due to chance ($P = 0{\cdot}26$).

DISCUSSION

Even in a widely contaminated environment with numerous opportunities for recontamination of washed hands, mothers who were given soap and encouraged to use it had measurably cleaner hands on unannounced follow-up visits than mothers who were not. In experimental settings where hands of study subjects were contaminated with coliform bacteria, washed with soap and water, dried, and immediately cultured, the concentration of bacteria decreased on average by two logs [17, 18]. In this Karachi squatter settlement, the hands of mothers who used soap had a mean 42–63% fewer organisms compared with their baseline measurements. This higher level of relative contamination than was found in experimental studies likely reflects reexposure to the heavy contamination of the environment and the delay between hand washing and unannounced culturing of the hands, rather than a failure of hand washing with soap to remove coliform bacteria from hands. In a study among Guatemalan street vendors, hands washed with soap and clean water were clean immediately after washing, but within 1 h they were as contaminated with *E. coli* and thermotolerant coliforms as the hands of vendors who had not washed their hands [19]. The difference in hand washing effectiveness among Karachi mothers may reflect a lower rate of hand recontamination in their households than occurred among vendors in the streets and markets of Guatemala city.

Hand contamination was worse during the third week of the intervention, the week corresponding with holiday preparations that require numerous additional responsibilities for mothers including cleaning the home, purchasing gifts, preparing for guests, and preparing special foods. The untreated drinking water quality in the third week was no higher than in the sixth or tenth week [14], suggesting that the higher hand contamination in the third week likely resulted from either less frequent hand washing or more frequent hand recontamination during a busy week.

Differences were observed in the level of hand contamination between mothers who were given soap and encouraged to use it and mothers who were not. The difference in the level of hand contamination between mothers who washed their hands with soap and chlorinated water compared with those who washed their hands with soap and the available heavily contaminated water was less and did not reach statistical significance. This finding contrasts with a study in Uttarkhan, Bangladesh where women who washed their hands with tubewell water, which had a mean contamination level of 32 faecal coliforms/100 ml, had less than half as many faecal coliform bacteria on their hands than did women who washed their hands with pond water, which had a mean of 17330 faecal coliforms/100 ml [6]. In Karachi, baseline drinking water samples among households receiving only soap had a mean of 10864 faecal coliforms/100 ml, similar to the Uttarkhan pond water. Although we did not quantify the thermotolerant coliforms in chlorinated vessel water used for hand washing, hypochlorite treatment and storage in the vessel reduced the concentration of thermotolerant colonies by > 99% in the drinking water in these same communities [14], that is, to a level similar to the tubewell water in Uttarkhan. The primary difference between these two studies is that in Uttarkahn, most of the women used soil rather than soap as a rubbing agent to wash their hands. Our data suggest that if hands are washed with soap, the quality of water may not be as important. Thus, where clean water is limited or expensive, and where soap is available, it may be best to encourage hand washing with soap and untreated water and reserve the cleanest water for drinking.

There are important limitations to this study. First, the three intervention groups represented three different neighbourhoods. The neighbourhoods differed in the amount of money spent on soap, the percentage of households with a direct municipal water connection, the level of maternal literacy, and their baseline hand contamination. Thus, it is possible that differences in outcomes among the groups reflected underlying differences in their neighbourhoods rather than the effects of the assigned interventions. However, we included neighbourhood characteristics likely to affect hand cleanliness and hand washing, in the general linear model, which adjusted for these differences and reenforced the conclusion of the univariate analysis that assignment to an intervention which included soap was associated with markedly cleaner hands.

A second limitation of the study is that a specimen of the water used to wash hands was not collected from each household each time a hand rinse specimen was collected. It is possible that such precise data would identify a significant effect of the level of bacterial contamination of water used to wash hands with soap on hand cleanliness. Similarly, since measurements of hand contamination are highly variable, it is possible that with a larger sample size a difference in hand cleanliness among persons using soap and cleaner water would be detected. However the limited data from this study suggest that the greatest difference in hand cleanliness is achieved through washing hands with soap, and that cleaning the water used for washing, would be expected, at best, to make a lesser contribution.

In this low-income Muslim community where soap was available and well accepted, intensive community-based promotion of hand washing and provision of additional soap produced a measurable improvement in microbiologic hand cleanliness. Intensive community-based efforts have successfully promoted hand washing in other low-income communities with a high incidence of diarrhoeal disease [2, 4, 5]. Indeed in two settings [2, 5] intensive community based efforts to promote hand washing with soap were effective in reducing diarrhoeal disease even when soap was not freely provided. Delivering intensive community-based hand-washing promotion campaigns to all such communities worldwide would require a substantial commitment of financial and human resources. A challenge for the public health community is to develop means of promoting hand washing which reach a large number of communities with a high incidence of diarrhoea at lower cost. In resource constrained settings, one option has been to develop partnerships between soap manufacturers who have regular advertising budgets and an interest in increased soap sales with public health organizations interested in promoting increased effective hand-washing [20, 21]. Research which measures the effectiveness of these co-sponsored interventions, the cost effectiveness of promoting hand washing alone, and the incremental cost benefit of adding hand washing promotion to other water and sanitation interventions would help to develop diarrhoea prevention initiatives with the greatest likelihood of global implementation.

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