

Field trial of a low cost method to evaluate hand cleanliness

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Summary

OBJECTIVE To evaluate a simple low cost method for measuring hand contamination as an objective assessment of handwashing practices.

METHOD As part of a larger randomized controlled trial of handwashing promotion with soap conducted in squatter settlements of Karachi, Pakistan, a randomly selected subset of 52 mothers in households receiving soap and handwashing promotion and 28 mothers in control households directly pressed three fingers of their right hand onto MacConkey agar plates on weekly unannounced visits from April to September 2002, and monthly from October 2002 to March 2003. The MacConkey plates were incubated at 44 °C for 24 h, and evaluated for growth of thermotolerant coliform bacteria.

RESULTS The proportion of samples that had detectable thermotolerant coliforms (50%) was similar in households that received soap and control households (52%, $P = 0.40$). In the week after evaluation of the mothers' hands, the proportion of households that reported diarrhoea was similar regardless of whether or not the mother had thermotolerant coliforms detected by direct finger imprint (18.6% *vs.* 19.1%, Relative Risk 0.99, 95% CI 0.96, 1.03).

CONCLUSIONS A three finger direct imprint test using MacConkey agar for thermotolerant coliforms was not a useful method to assess regular handwashing practices with soap in Karachi. Developing better measures of handwashing behaviour remains an important research priority.

keywords handwashing, soap, hand contamination

Introduction

Diarrhoea and respiratory disease are leading global causes of death in early childhood (Bryce *et al.* 2005). Focused intervention studies consistently demonstrate that handwashing with soap reduces diarrhoea and respiratory disease (Curtis & Cairncross 2003, Luby *et al.* 2005, Rabie & Curtis 2006). An important difficulty in translating handwashing promotion interventions that have been effective in controlled small-scale studies into large-scale intervention programs is measuring handwashing behaviour. Responses to questions about hand washing are substantially different from observed behaviour (Manun'Ebo *et al.* 1997). Structured observations have also been used to assess frequency and appropriateness of handwashing (Curtis *et al.* 2001). However, structured observations have problems with both validity and logistics. The threat to validity is that the presence of an observer would be expected to change behaviour especially if an intervention had suggested that handwashing was expected and a study investigator came into the home to

observe. The logistical difficulty is that structured observations are time consuming and expensive and so are used sparingly, if at all, in assessing a handwashing promotion intervention. The end result is that we do not know how effectively broad efforts to promote handwashing actually improve the rate of handwashing and hand cleanliness (Luby 2001). This lack of clarity on intervention effectiveness translates into tepid support for handwashing promotion efforts, and when funded, uncertainty about how best to improve hand hygiene.

An alternative approach to evaluating handwashing intervention efforts would be to evaluate hand microbiology, specifically the presence on mother's hand of organisms whose normal environment is the human gastrointestinal tract. Various approaches have been used to assess hand contamination (Kaltenthaler & Pinfold 1995). In research studies in communities with a high incidence of diarrhoea, samples collected from hand swabs (Han *et al.* 1986), water used to wash hands (Kaltenthaler *et al.* 1991) and from sterile solution that hands are rinsed in (Pinfold 1990, Luby *et al.* 2001) have been quantitatively analysed using

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methods similar to assessing water quality. However, these approaches are expensive and complex enough that they would be difficult to use widely for program evaluation.

A simpler lower cost approach to assess hand contamination is direct finger impression onto selective agar (Kaltenthaler *et al.* 1995). The detection of thermotolerant coliforms on hands by direct impression was associated with diarrhoea incidence among children in one low income community in Bangladesh (Henry & Rahim 1990) and among children in day care centres in the United States (Ekanem *et al.* 1983, Van *et al.* 1991).

We evaluated direct finger impression onto selective agar as a simple low cost method for evaluating hand contamination with fecal organisms; we assessed its association with household handwashing practices and diarrhoea.

Methods

This evaluation was part of a larger study that has been described previously (Luby *et al.* 2004). In short, 36 low-income squatter settlement neighborhoods in Karachi, Pakistan were randomly assigned to either soap with handwashing promotion or control. Six-hundred households received either antibacterial or plain soap and 306 control households received school supplies. Households were visited at least weekly for 51 weeks to evaluate health outcomes, and, in intervention households, to encourage handwashing with soap at key times—after defecation, after cleaning an infant who had defecated and before preparing food.

We used a random number generator to select 80 of the households enrolled in the larger study to provide repeated measures of hand contamination. The mothers of the children aged under 5 years who were enrolled in the study were asked to provide specific informed consent for sample collection to assess hand contamination. Households were not notified in advance that hand samples would be collected on that day. If the mother's hand was visibly soiled because she was preparing food or cleaning immediately before the visit, then the sample was collected at another time.

Field workers brought MacConkey agar plates to selected households. Mothers were asked to press the second, third, and fourth finger of their right hand onto the agar plate and hold it there for 5 s. The study team transported the plates to a commercial microbiology laboratory. Laboratory personnel incubated the plates at 44 °C for 24 h, then counted the number of colony forming units (cfu) on the plate. Direct finger imprint samples were collected from consenting mothers weekly from April to September 2002, and monthly from October 2002 to March 2003.

To compare the direct finger imprint method to a more sensitive method, for every twenty-fifth direct finger imprint sample, a hand rinse sample was also collected. For the quantitative hand rinse sample, the Aga Khan University Hospital laboratory prepared sterile 1-l sample collection bags (Whirl-Pack® Nasco, Fort Atkinson, WI,) containing 125 ml of 0.1% peptone broth. The study team asked the mother to insert one hand at a time into the sterile collection bag and gently shake each hand for 5 s to rinse it thoroughly in the peptone broth. The study team placed the broth samples in a cooler on ice and transported them to the Aga Khan University Hospital Laboratory for analysis within 4 h of collection. Laboratory personnel diluted the broth at 1:10, 1:100 and 1:1000 dilution with 0.01 M sterile phosphate buffered saline. They filtered 50 ml of each dilution through a sterile 0.45- μ m paper filter, and transferred each filter to a 60-mm petri plate containing EMB agar. Laboratory personnel incubated the plates at 44 °C for 24 h and counted the number of colonies visible with each dilution.

For each mother, the proportion of samples with at least one cfu of thermotolerant coliforms was calculated and the mean proportion of hands contaminated with thermotolerant coliforms was compared between households receiving soap and control households with a *t*-test. Geometric means (log base 10) were used to compare the results of hand rinse samples because the numbers are calculated based on results of dilutions that differ by an order of magnitude. As triclocarban, the active agent in the antibacterial soap is not active against Gram-negative bacteria, we analysed all of the soap households together.

We assessed the proportion of households with a new episode of diarrhoea reported in the week after the detection of thermotolerant coliforms by direct finger imprint of a mother's hands with the proportion of households with new episodes of diarrhoea where the mother's hands were not contaminated by calculating relative risks and confidence limits. We compared continuous variables with the *t*-test if the underlying distributions were normal and the Wilcoxon rank sum test if they were non-normal to determine if the distributions differed more than would be expected by chance.

The study protocol was approved by the ethics review committee of the Aga Khan University and an institutional review board of the Centers for Disease Control and Prevention.

Results

The 80 randomly selected households included 52 households randomized to receive soap and handwashing promotion and 28 control households. The household

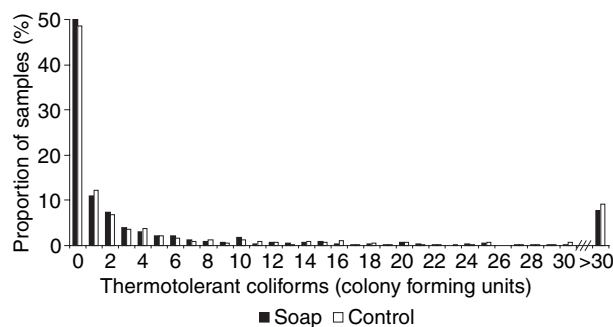
Table 1 Baseline household characteristics among mothers whose hands were repeatedly sampled, Karachi, Pakistan 2002

	Soap (<i>n</i> = 52)	Control (<i>n</i> = 28)
Persons per household (mean)	10.4	11.1
Children < 5 years of age per household (mean)	1.8	1.7
Rooms in house (mean)	2.1	2.5
Households with infants (%)	19	25
Father of the youngest child is literate (%)	52	71
Mother of the youngest child is literate (%)	42	29
Monthly household income < 60 US\$ (%)	50	71
Refrigerator ownership (%)	35	43
Primary drinking water source		
Municipal supply within the house (%)	15	7
Municipal supply at a community tap (%)	13	14
Tanker truck (%)	60	57
Water bearer (%)	6	21
Other (%)	6	0
Toilet without flush tank in the home (%)	94	100
Handwash station with soap and water seen by study workers (%)	98	93
Faeces visible where children have access (%)	92	79
Kitchen judged by field worker to be dirty (%)	2	7

characteristics were broadly similar in handwashing promotion with soap *vs.* control households (Table 1).

Field workers collected 2 485 samples from 80 households. The distribution of cfu of thermotolerant coliforms from direct finger imprint specimens was skewed. 1 230 samples (49%) had no detectable thermotolerant coliforms, 1 050 samples (42%) had between 1 and 30 cfu of thermotolerant coliforms and 205 (8%) had >30 cfu. The distribution of values from mothers living in households that received soap and handwashing promotion was similar to the distribution from mothers living in control households (Figure 1).

The proportion of samples that had detectable thermotolerant coliforms was similar in households that received

**Figure 1** Proportion of hand imprint samples from mothers in handwashing promotion and control households by number of colony forming units of thermotolerant bacteria detected (*n* = 2485).

soap and control households (Table 2). The proportion of hands contaminated varied by week, and was somewhat higher in April through October than November through March (Figure 2).

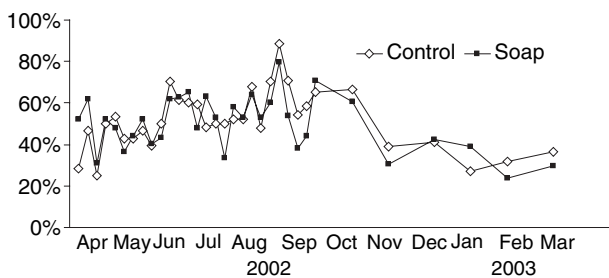
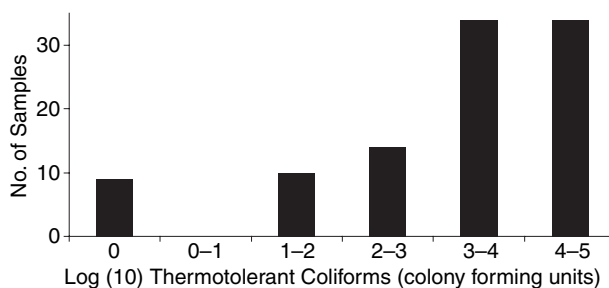
In the week following evaluation of the mother's hands, the proportion of households that experienced diarrhoea and where the mother had thermotolerant coliforms detected by direct finger imprint was similar to households where the mother had no detectable thermotolerant coliforms on hand contamination (18.6% *vs.* 19.1%, Relative Risk 0.99, 95% CI 0.96, 1.03).

The longitudinal prevalence of diarrhoea, that is the percentage of person-weeks with diarrhoea over the 51-week study period, in the 21 households whose mothers' hands repeatedly tested cleanest (detectable thermotolerant coliforms 22–42% of tested samples) was lower than in the 22 households whose mothers hands were the most frequently contaminated (detectable thermotolerant coliforms on 58–78% of sample tested) although the difference was not statistically significant (2.9% *vs.* 3.8%, *t*-test *P* value = 0.31).

We collected 101 hand rinse samples; 60 (75%) of the mothers contributed at least one. Of these 101 samples, 92 (91%) had detectable thermotolerant coliforms and 20 (20%) had detectable *Escherichia coli*. There was no difference in the geometric mean level of contamination with thermotolerant coliforms measured by hand rinse between households that received handwashing promotion with soap and control households (3.57 *vs.* 3.53 *P* = 0.86). Both the direct finger imprint assessment and the hand

Table 2 Proportion of households with hand contamination by receipt of supplemental soap

	Number of households	Number of samples	Mean proportion of household samples with > 0 thermotolerant coliforms	Mean proportion of household samples with > 30 thermotolerant coliforms	Mean thermotolerant coliforms	Median thermotolerant coliforms
Soap	52	1638	50%	7.6%	8.8	1
Control	28	847	51%	9.0%	10.0	1
<i>t</i> -test <i>P</i> -value			0.57	0.31	0.20	

**Figure 2** Proportion of hand samples with detectable thermotolerant coliform bacteria by week, Karachi, Pakistan, April 2002–March 2003.**Figure 3** Distribution of hand contamination with thermotolerant coliforms among hand rinse samples ($n = 101$).

rinse assessment measured thermotolerant coliforms, but the hand rinse samples counted many more organisms. The 101 hand rinse samples had a median 4 000 cfu of thermotolerant coliforms per 100 ml, compared with a median 0 cfu by direct finger imprint. The distribution of thermotolerant coliform contamination as measured by hand rinse was skewed to the left (Figure 3) in contrast to the measurements by direct imprint method, which were skewed to the right (Figure 1).

Setting the hand rinse assessment as the standard, the direct finger imprint assessment was 48% sensitive, and 75% specific. The mean log most probable number of

thermotolerant coliforms on hand rinse testing was similar among hands that had thermotolerant coliforms detected on the direct finger imprint testing and hands with no detected thermotolerant coliforms on direct finger imprint testing (3.66 *vs.* 3.48, $P = 0.37$).

We assessed the relationship between hand rinse measurements and diarrhoea. We divided hand rinse samples into those that were above the median (4000 cfu of thermotolerant coliforms per 100 ml) and those below the median. In the week after the sample was collected, households in which the mother's hand rinse specimen were above the median level of contamination were 2.9 times more likely to report a child with diarrhoea than households whose mother's hands were below the median level of contamination (29% *vs.* 10%, 95% CI 1.1, 7.3). When the analysis was restricted to only those households that reported no diarrhoea in the week the sample was collected ($n = 83$), households in which the mother's hand rinse specimens were above the median level of contamination were 1.9 times more likely to report a child with diarrhoea than households in which the mother's hands were less contaminated, although chance could not be excluded as the explanation for the difference (18% *vs.* 9%, 95% CI 0.60, 5.9).

During the 100 household weeks of observations that followed collection of the hand rinse sample, children living in households that received soap reported much less diarrhoea than households that did not receive soap (10.9% *vs.* 2%, $P = 0.001$). Similarly during the 2 376 household weeks of observation that followed collection of the direct imprint sample, children living in households that received soap reported less diarrhoea than children in households that did not receive soap (6.2% *vs.* 3.1%, $P < 0.001$).

Discussion

Hand contamination as assessed by the direct three finger imprint test using MacConkey agar for thermotolerant coliforms was not associated with relevant exposures

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(receiving soap and handwashing promotion) or outcomes (diarrhoeal disease). In the larger intervention trial households randomized to soap and handwashing promotion received three times as much soap as control households reported purchasing (Luby *et al.* 2004), yet we measured no difference in the proportion of mothers' hands that were contaminated in households that received soap compared with control households. Moreover, when thermotolerant coliforms were detected on a mother's hands, her household was no more likely to report a new episode of diarrhoea in the following week than households where no thermotolerant coliforms were detected on the mother's hands. The direct finger imprint tests were not associated with the results of the quantitative assessment of hand rinse specimens. Taken together, these results suggest that the direct three finger imprint test using MacConkey agar for thermotolerant coliforms is not a useful method to assess regular handwashing in Karachi.

By contrast the hand rinse specimens do appear to be a valid measurement of contamination. Hand contamination of mothers as measured through hand rinse was associated with diarrhoea among children in the household in the subsequent week. Similarly, those households that received soap and handwashing promotion had a lower prevalence of diarrhoea. However, hand contamination as measured by hand rinse was not associated with receiving soap and handwashing promotion. This suggests that both receipt of soap and thermotolerant coliforms measured by hand rinse on unannounced visits each assessed a relevant dimension for diarrhoea prevention, but they are fairly independent of each other. Thus, hand rinse specimens for thermotolerant coliforms was not an efficient method to assess handwashing behaviour.

The lack of association between thermotolerant coliforms as detected by finger imprint and subsequent diarrhoea contrasts with results from other settings. In one community in Bangladesh, (but not in another) hand contamination measured by finger imprint on two consecutive days in mid-June was associated with incidence of diarrhoea among children under the age of 6 years over an entire year (Henry & Rahim 1990). This suggests that hand contamination on the two days tested was associated with general environmental contamination and so diarrhoea throughout the year. In day care centres in Houston, hands were more likely to be contaminated when children in the same room had diarrhoea in the same week, although it's not clear if the hand contamination resulted from the diarrhoea or caused it (Van *et al.* 1991). In both Bangladesh and Houston direct impression samples were collected from both hands and from all fingers and the palms. In Houston, study subjects kept their hands in contact with the agar for 15 s. By contrast in Karachi to

lower cost and to simplify collection we used small agar plates and collected samples from only three fingertips of a single hand maintaining contact with the agar for 5 s. These results with larger agar plates and hand rinse specimens suggest that culturing a larger surface area of the hand may provide a more accurate and useful assessment of thermotolerant coliform contamination.

One problem with using microbiological methods of hand cleanliness is that in highly contaminated environments hands that are effectively decontaminated with handwashing are often quickly recontaminated (Sobel *et al.* 1998). Thus, unannounced evaluations of hand cleanliness may not efficiently capture improved handwashing with soap and key times. A second problem with hand rinse samples is that the results are highly variable, and so likely have many determinants other than frequency of handwashing with soap. In a prior study in Karachi squatter settlements, mothers who received soap and handwashing promotion had 65%–74% fewer thermotolerant coliforms from hand rinse specimens collected on unannounced visits compared with mothers who received no hand hygiene intervention (Luby *et al.* 2001). This prior analysis was based upon 232 samples that were adjusted based on a pre-intervention baseline measurement. In the present study, only 101 hand rinse specimens were collected. There was no pre-intervention baseline. Highly variable measures require large sample sizes to assess relationships, and thus are not efficient ways to objectively classify households into those practicing effective handwashing with soap and those that do not.

Pinfold and colleagues used a larger KF *Streptococcus* agar plate and had study subjects press all 10 fingers on one plate. They collected plates from at least three family members, constructed an index of the mean fingertip counts and reported a difference in the hand contamination index in households receiving a handwashing intervention (Pinfold & Horan 1996). In the present study, we chose not to use faecal streptococci as an indicator because of concern that the triclocarban in the antibacterial soap might differentially affect the indicator organism, but not have an effect on the organisms most likely to cause diarrhoea. However, further evaluation of faecal streptococci as a handwashing indicator should be considered.

There are important limitations to this study. First, this approach was attempted in one place, using direct imprint of 3 fingertips and a single commercial laboratory. It is possible that in other settings with a larger surface area of the hand tested, the approach may be more successful. A second limitation is that the assessment of handwashing with soap is imperfect. Handwashing was not observed in households that received soap. Thus, it is possible, that the direct finger imprint test accurately assessed similar

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levels of hand contamination and similar levels of soap use. However, soap supply was routinely checked and routinely available in households that received soap and handwashing promotion. There was no evidence that soap was sold or used for other purposes in intervention households. In addition the direct finger imprint test results were not associated with quantitative hand rinse results. Thus, it is most likely that the similar results in soap and intervention households reflects a difficulty with the measurement of hand contamination.

A valid low-cost approach to assess the frequency of handwashing with soap would translate into an improved capacity to evaluate and so improve handwashing promotion interventions. The specific approach used in this study, direct three finger imprint on MacConkey agar, did not effectively identify persons who washed their hands with soap more regularly. Developing better measures of handwashing behaviour remains an important research priority.

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S. P. Luby *et al.* **Evaluating Hand Cleanliness****Etude sur le terrain d'une méthode peu coûteuse pour évaluer la propreté des mains**

OBJECTIF Evaluer une méthode simple et peu coûteuse pour mesurer la contamination des mains pour servir comme évaluation objective des pratiques de lavage des mains.

MÉTHODE Cette étude est un élément d'une plus grande étude randomisée contrôlée sur la promotion du lavage des mains au savon, menée dans des zones de squats d'habitations à Karachi au Pakistan. Un sous-groupe de 52 mères aléatoirement choisies dans les familles recevant la promotion du savon et du lavage des mains et 28 mères dans les familles contrôles ont directement pressé trois doigts de leur main droite sur des boîtes d'agar de MacConkey, au cours de visites inattendues hebdomadaires à partir d'avril jusqu'à septembre 2002 et de visites mensuelles d'octobre 2002 à mars 2003. Les boîtes de MacConkey ont été incubées à 44 °C pendant 24 heures et examinées pour la croissance de bactéries coliformes thermo tolérantes.

RÉSULTATS La proportion d'échantillons avec des coliformes thermo tolérants discernables était similaire dans les familles qui ont reçu le savon comparées aux familles contrôles (50% contre 52%, $P = 0,40$). Dans la semaine suivant l'évaluation des mains des mères, la proportion de familles qui ont rapporté de la diarrhée était similaire quelque soit le résultat de coliformes thermo tolérants observés chez les mères sur base de l'impression directe des doigts (18,6% contre 19,1%, risque relatif 0,99; IC95%: 0,96–1,03).

CONCLUSIONS Le test direct de l'impression de trois doigts sur l'agar de MacConkey pour les coliformes thermo tolérants n'était pas une méthode utile pour évaluer les pratiques de lavage régulier des mains avec du savon à Karachi. Le développement de meilleures mesures de comportement de lavage de mains reste une priorité importante de recherche.

mots clés lavage des mains, savon, contamination des mains

Ensayo de campo de un método de bajo coste para evaluar la limpieza de las manos

OBJETIVO Evaluar un método simple y de bajo coste para medir la contaminación de las manos como una valoración objetiva de las prácticas de lavado de manos.

MÉTODO Como parte de un ensayo más grande, controlado y aleatorizado, de promoción del lavado de manos con jabón, realizado en asentamientos ilegales de Karachi, Pakistan, se seleccionó de forma aleatoria un subgrupo de 52 madres entre las de hogares que recibían jabón y promoción del lavado de manos, y 28 madres del grupo control. Todas presionaron, con tres dedos de su mano derecha, sobre placas de agar MacConkey, durante visitas semanales no anunciadas entre Abril – Septiembre del 2002, y de forma mensual entre Octubre 2002 – Marzo 2003. Las placas de MacConkey fueron incubadas a 44 °C durante 24 horas, y evaluadas para el crecimiento de bacterias coliformes termotolerantes.

RESULTADOS La proporción de muestras que tenían coliformes termotolerantes detectables fue similar en los hogares que recibieron jabón, comparados con los hogares control (50% versus 52%, $P = 0.40$). Durante la semana después de la evaluación de las manos de la madre, la proporción de hogares que reportaron diarrea fue similar, independientemente de si en la madre se habían detectado o no coliformes termotolerantes con la impronta directa del dedo (18.6% versus 19.1%, Riesgo Relativo 0.99, 95% CI 0.96, 1.03)

CONCLUSIONES Una prueba de impronta de tres dedos utilizando agar MacConkey para coliformes termotolerantes no fue un método útil a la hora de evaluar la práctica regular del lavado de manos con jabón en Karachi. El desarrollar mejores medidas para el lavado de manos continúa siendo una prioridad de investigación.

palabras clave lavado de manos, jabón, contaminación manos