Efficacy of alcohol-based hand sanitizer on hands soiled with dirt and cooking oil

Amy J. Pickering, Jennifer Davis and Alexandria B. Boehm

ABSTRACT

Handwashing education and promotion are well established as effective strategies to reduce diarrhea and respiratory illness in countries around the world. However, access to reliable water supplies has been identified as an important barrier to regular handwashing in low-income countries. Alcohol-based hand sanitizer (ABHS) is an effective hand hygiene method that does not require water, but its use is not currently recommended when hands are visibly soiled. This study evaluated the efficacy of ABHS on volunteers' hands artificially contaminated with *Escherichia coli* in the presence of dirt (soil from Tanzania) and cooking oil. ABHS reduced levels of *E. coli* by a mean of 2.33 log colony forming units (CFU) per clean hand, 2.32 log CFU per dirt-covered hand, and 2.13 log CFU per oil-coated hand. No significant difference in efficacy was detected between hands that were clean versus dirty or oily. ABHS may be an appropriate hand hygiene method for hands that are moderately soiled, and an attractive option for field settings in which access to water and soap is limited.

Key words | alcohol-based hand sanitizer, antimicrobial efficacy, *Escherichia coli*, hand hygiene, soil

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INTRODUCTION

Hand hygiene is an effective strategy for reducing the global burden of infectious disease, particularly respiratory and gastrointestinal illnesses (Aiello *et al.* 2008). Clean hands prevent the spread of pathogens via the fecal–oral route, including the transmission of pathogens from hands to food and drinking water (Curtis & Cairncross 2003). Previous research has documented that fecal bacteria levels in stored drinking water are positively associated with fecal bacteria levels on household members' hands (Pickering *et al.* 2010b), and that home drinking water storage containers allowing hand-water contact are associated with increased incidence of diarrheal disease (Trevett *et al.* 2005). Diarrhea is one of the leading causes of childhood mortality, and handwashing with soap has been found to reduce episodes of diarrhea by an average of 30% (Ejemot *et al.* 2008). Although soap is widely accessible around the world, there is evidence that adoption of regular handwashing can be affected by limited and unreliable quantities of available water supplies (Tumwine *et al.* 2002; Wolf-Peter *et al.* 2009).

Alcohol-based hand sanitizers (ABHS) are an alternative to handwashing with soap that do not require water. They have been found to improve hand hygiene compliance, and to significantly reduce the rate of infection, in health care settings (Widmer 2000; Fendler *et al.* 2002; Mody *et al.* 2003). Several laboratory studies have demonstrated that ABHS can reduce bacterial test organisms such as *Escherichia coli*, *Staphylococcus aureus*, and *Serratia marcescens* by greater magnitudes than soap and water (Bloomfield *et al.* 2007). ABHS efficacy has also been shown to be equal or better than handwashing with soap at removing bacterial contamination in field settings among veterinary staff in Canada (Traub-Dargatz *et al.* 2006), livestock handlers in the US (Davis *et al.* 2006), and mothers visiting a health clinic in Tanzania (Pickering *et al.* 2010a). Previous research suggests that ABHS is not as effective as handwashing with soap at removing spore forming bacteria such as *Clostridium difficile* from hands (Oughton *et al.* 2009).

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Both the World Health Organization (WHO) and the Centers for Disease Control and Prevention recommend that ABHS not be used when hands are visibly soiled because ABHS does not remove dirt or other substances from the hands (CDC 2002; WHO 2006). Alcohol in hand sanitizers disinfects hands by penetrating the cell membranes of pathogens and denaturing proteins. There is concern that organic substances on soiled hands can inhibit antimicrobial activity of hand sanitizer by shielding pathogens from alcohol exposure (WHO 2006). However, there is no published evidence indicating that ABHS antimicrobial efficacy is inferior to handwashing with soap on soiled hands. Existing laboratory studies have found that the presence of proteins (e.g. fetal calf serum) on hands does not significantly affect the efficacy of ABHS against bacteria and viruses (Kampf *et al*. 2002; Sickbert-Bennett *et al*. 2004; Rabenau *et al*. 2005). ABHS has also been found to be better at disinfecting hands than handwashing with soap and water when blood is present (Larson & Bobo 1992), but there are no known studies investigating the shielding effect of materials more commonly found on the hands of non-health care workers, such as food, dirt, and oil.

This research is motivated by the facts that compliance with hand hygiene regimens suffers when water and/or soap are not easily accessible, and that scarcity of handwashing supplies is regularly experienced by the 80% of the world's population living in low-income countries. As a result, the hands of people in low-income countries are regularly soiled with substances such as dirt (soils from gardening or farming), charcoal, and oil. Identifying hand hygiene strategies that are effective in removing disease causing microorganisms but do not require substantial inputs of water could thus have health benefits for households in low-income countries. This study investigates the antimicrobial efficacy of ABHS on hands soiled with dirt, and on hands covered in cooking oil, as compared to the antimicrobial efficacy of ABHS on visibly clean hands.

METHODS

Data were collected during April and May (2008) with 15 subjects at Stanford University. Participants were Stanford graduate and undergraduate students voluntarily recruited to participate following appropriate informed consent procedures approved by the Stanford Human Research Protection Program. To assess ABHS efficacy, hands were inoculated with a non-virulent strain of *E. coli* (FDA strain Seattle 1948, ATCC# 25922) and sampled before and after the use of ABHS. All participants performed three separate efficacy tests, in which ABHS efficacy was evaluated on (1) clean hands; (2) hands covered with sterile dirt; and (3) hands covered in cooking oil. Each participant was randomized to one of six possible treatment orders: (a) dirt, oil, clean; (b) oil, clean, dirt; (c) clean, oil, dirt; (d) dirt, clean, oil; (e) oil, dirt, clean; (f) clean, dirt, oil.

Upon arrival and prior to each efficacy test, the subject washed his or her hands with non-antimicrobial soap (Ivory, Proctor and Gamble, Cincinatti, OH) and tap water. The subject wet his/her hands, then two pumps of soap were placed on the hands and the subject was asked to follow a diagram of motions recommended by the WHO for correct handwashing with soap [p. 101, *WHO Guidelines on Hand Hygiene in Health Care* (WHO 2006)] After hands were rinsed free of soap, they were dried thoroughly with clean paper towels.

Hands were inoculated by having the subject rub 1 mL of *E. coli* suspended at a concentration of 10^7/mL in Tryptic Soy Broth (BD, Franklin Lakes, NJ) over all surfaces of their hands until visibly dry. For dirt and oil efficacy tests, dirt or oil was applied after the hands had been inoculated with *E. coli*. Pre-weighed vials of dirt were prepared containing 0.2 g of soil that had been collected from Tanzania, transported to Stanford University, and autoclaved. The dirt was applied to one palm of the subject's hand and he/she was asked to rub his/her hands together to work the dirt into the skin on the palms and fingers. For the cooking oil efficacy test, 0.5 mL of corn oil (ConAgra, Memphis, TN) was dispensed onto the palm of one hand using a Repipet II Dispenser (Thermo Scientific, Waltham, MA). The subject then rubbed both hands together to coat both hands with oil.

One hand was selected by a coin toss (heads: right, tails: left) and a 'before' hand rinse sample was obtained. Next, the subject was instructed in correct use of ABHS following the WHO recommended motions [p. 100, *WHO Guidelines on Hand Hygiene in Health Care* (WHO 2006)]. The sequence of motions included rubbing: palm to palm; right

palm over left dorsum with interlaced fingers and vice-versa; palm to palm with fingers interlaced; backs of fingers to opposing palms with fingers interlocked rotational rubbing of left thumb clasped in right palm and vice-versa; and rotational rubbing backwards and forwards with clasped fingers of right hand in left palm and vice-versa. Each ABHS use consisted of placing 2 mL of gel distributed evenly on all areas of both hands until completely dry. Subsequently, an 'after' hand sample was obtained from the subject's *other* hand (based on the assumption that the hand sampled before use of ABHS would be less contaminated as a result of the sampling method) (Pickering *et al*. 2010a).

After the hand sampling was complete, participants were questioned regarding their perception of ABHS efficacy under the three treatment conditions: clean, dirty, and oily. Specifically participants were asked 'How effective do you think the hand sanitizer was in removing the bacteria from your hands in the following situations: clean, dirt, and oil?' Answer choices included '100% effective', 'mostly', 'somewhat', 'slightly' and 'not at all'.

Hand sampling was conducted using the hand rinse method, during which the hand is placed in a 69 oz Whirl-Pak (NASCO, Fort Atkinson, WI) bag filled with 350 mL of sterile water for 60 s (30 s of shaking and 30 s of hand massage through the bag) (Pickering *et al*. 2010a). All hand rinse samples were kept on ice and processed by membrane filtration within 4 h of collection. Each sample was passed through a 47-mm-diameter 0.45-μm cellulose filter (Millipore Inc., Billerica, MA) and then placed on MI media (BD Difco, Franklin Lakes, NJ) to culture *E. coli*. Samples were incubated at $35 \pm 0.5\,^{\circ}C$ for 24 h according to the United States Environmental Protection Agency method 1604 (USEPA 2002).

At least two volumes were filtered for each sample; dilutions were performed with sterile water in order to filter volumes less than 10 mL. Samples obtained before use of ABHS were filtered at volumes of 0.01 and 0.1 mL, giving a lower detection limit of 3.5×10^3 colony forming units (CFU) per two hands and upper limit of 1.75×10^7 CFU per two hands. Samples obtained after use of ABHS were filtered at volumes of 1 and 10 mL, giving lower and upper detection limits of 35 CFU per two hands and 1.75×10^5 CFU per two hands, respectively. Only results within the detection limits were used in the analysis. If both volumes for a sample gave results that were within the detection limits, these results were averaged together for the analysis.

All results were $\log_{10}$ transformed for analysis. Paired *t*-tests were used to calculate mean log-reduction of *E. coli* after use of ABHS under the three different treatment conditions. Paired *t*-tests were also conducted to detect significant differences in log-mean reductions by ABHS on clean versus dirt-covered and clean versus oil-coated hands. *P*-values below 0.05 are considered statistically significant.

RESULTS

Study volunteers included 10 males and 5 females, with a mean age of 28 years (standard deviation (SD) 4.5), and all were right handed. Volunteers reported washing their hands a mean of six times per day (SD 4, median 5), and most reported using hand sanitizer only when handwashing facilities were not available (66%) or never having used hand sanitizer (27%).

The use of ABHS significantly reduced the levels of *E. coli* recovered from subject's hands for all three treatments by similar magnitudes (Table 1). Paired sample *t*-tests of levels of *E. coli* before and after the use of ABHS found mean log reductions of 2.33 (standard error (SE) 0.2), 2.32 (SE 0.2), and 2.13 (SE 0.1) log CFU/hand on clean hands, hands with dirt, and hands with oil, respectively. However, no significant difference in mean log reduction or mean percent log reduction was found between clean *versus* soiled hands (paired *t*-tests, all $P > 0.3$) (Figure 1).

Almost all participants (93%) felt that ABHS was either 100% effective or mostly effective at removing the bacteria

Table 1 | Reduction in *E. coli* after use of ABHS by treatment type

Treatment	Log-mean reduction CFU/hand	95% CI of mean log reduction	Median % reduction (CFU/hand)	*N*	*P-value*
Clean	2.33	1.90–2.76	99.5	15	<0.001
Dirt	2.32	1.92–2.72	99.4	15	<0.001
Oil	2.13	1.87–2.37	99.3	15	<0.001

P-values calculated from paired *t*-test between before and after concentrations within subjects.

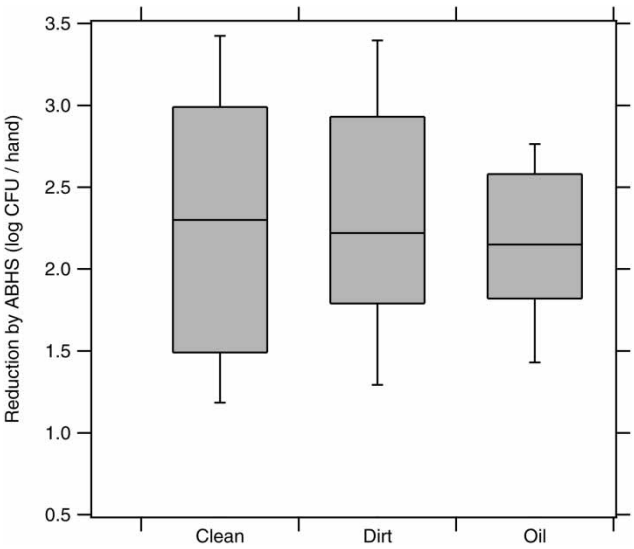

Figure 1 | Box and whisker plots of log-mean reduction in log CFU *E. coli* per hand after use of ABHS for each treatment group ($N = 15$ per treatment group).

from their clean hands. This percentage dropped to 67% for dirty hands, and to 40% for oily hands. None reported that ABHS was 'not at all' effective at removing bacteria for any of the treatment conditions.

DISCUSSION

ABHS significantly reduced levels of *E. coli* on hands in the presence of dirt and cooking oil by a magnitude similar to that observed on unsoiled hands. This finding stands in contrast to CDC and WHO published guidelines, which advise against use of ABHS on visibly dirty hands. In situations when hands are visibly dirty, the use of ABHS may be an appropriate and effective way to disinfect hands. Further work should be conducted to evaluate the efficacy of ABHS against other types of fecal indicator bacteria and viruses on soiled hands, as well as directly compare ABHS efficacy to the efficacy of handwashing with soap on soiled hands.

It is important to acknowledge that this was a study conducted in a laboratory setting, where visibly dirty and visibly oily hands were simulated by the application of these materials to subject's hands. These results may not represent the level of efficacy that would be achieved by ABHS on hands naturally soiled with dirt or oil. In fact, the bacteria reductions on clean hands detected in this work were much greater than what has been observed in field studies. In Dar es Salaam, Tanzania, Pickering *et al*. (2010a) observed a log-mean reduction of only 0.66 CFU *E. coli* per two hands among mothers using the same ABHS product and the same microbial sampling methods, whereas this study detected reductions greater than 2 log units.

Bacteria grown in the laboratory may attach to skin differently and be more susceptible to inactivation by ABHS as compared to natural flora. This work indicates that the efficacy of hand hygiene agents on artificially contaminated hands does not accurately represent efficacy against hand flora under field conditions, and suggests that efforts should be made to test hand hygiene agents on 'naturally contaminated' hands in order to determine true efficacy.

Participants perceived the efficacy of ABHS to be greater on their visibly clean hands compared to their dirty and oily hands. This lack of confidence in ABHS efficacy on soiled hands may be a potential behavioral barrier to ABHS use when hands are not visibly clean. However, all participants thought ABHS had at least some efficacy on their dirty and oily hands, which could translate into willingness to use ABHS on soiled hands when handwashing facilities are unavailable. Notably, perceptions of ABHS efficacy under laboratory conditions may be different from use in a field setting.

CONCLUSION

The presence of dirt and oil in visible amounts on hands does not appear to have a significant shielding effect on the antimicrobial efficacy of ABHS against the fecal indicator bacteria *E. coli*. ABHS may be a useful strategy to make hands safer in conditions of soiling, especially when water and soap is not readily accessible, a situation common to developing country settings.

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