Quantifying the Effect of Hand Wash Duration, Soap Use, Ground Beef Debris, and Drying Methods on the Removal of Enterobacter aerogenes on Hands

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ABSTRACT

Hand washing is recognized as a crucial step in preventing foodborne disease transmission by mitigating cross-contamination among hands, surfaces, and foods. This research was undertaken to establish the importance of several key factors (soap, soil, time, and drying method) in reducing microorganisms during hand washing. A nonpathogenic nalidixic acid–resistant Enterobacter aerogenes surrogate for Salmonella was used to assess the efficacy of using soap or no soap for 5 or 20 s on hands with or without ground beef debris and drying with paper towel or air. Each experiment consisted of 20 replicates, each from a different individual with ~6 log CFU/ml E. aerogenes on their hands. A reduction of 1.0 ± 0.4 and 1.7 ± 0.8 log CFU of E. aerogenes was observed for a 5-s wash with no soap and a 20-s wash with soap, respectively. When there was no debris on the hands, there was no significant difference between washing with and without soap for 20 s (P > 0.05). Likewise, there was no significant difference in the reductions achieved when washing without soap, whether or not debris was on the hands (P > 0.05). A significantly greater reduction (P < 0.05) in E. aerogenes (0.5 log CFU greater reduction) was observed with soap when there was ground beef debris on the hands. The greatest difference (1.1 log CFU greater average reduction) in effectiveness occurred when ground beef debris was on the hands and a 20-s wash with water was compared with a 20-s wash with soap. Significantly greater (P < 0.05) reductions were observed with paper towel drying compared with air (0.5 log CFU greater reductions). Used paper towels may contain high bacterial levels (>4.0 log CFU per towel) when hands are highly contaminated. Our results support future quantitative microbial risk assessments needed to effectively manage risks of foodborne illness in which food workers’ hands are a primary cause.

Hand washing is recognized as a crucial step in preventing foodborne disease transmission, by mitigating cross-contamination among hands, surfaces, and foods. It is considered a significant point of control for enteric pathogen transmission, especially for individuals who are shedding the pathogens asymptomatically (12, 15, 16, 18, 24, 27, 37, 41). The U.S. Food and Drug Administration (FDA) Model Food Code recommends washing hands at several occasions during food preparation (44). This includes, but is not limited to, before starting a food service task, in between handling ready-to-eat and non–ready-to-eat foods, after using the lavatory, and after handling soiled dishes or equipment. The FDA Model Food Code recommends washing hands for 20 s, under warm running water, with soap, and using either single-use towels or a forced air dryer to dry hands. Although the factors that influence hand washing effectiveness have been studied, these studies may not be comparable due to methodological differences (10) or statistical flaws (11, 17, 26). Evidence of the efficacy of air drying versus paper towel drying is contradictory. Some studies show that air drying is more effective (2), others show that towel drying is more effective (36, 42), and some show no difference (7, 17).

Microorganism concentration on a hand can vary from 10² to 10⁶/cm² depending on skin condition, whether or not the individual has recently handled raw foods, and frequency of hand washing (21, 29). The resident flora of skin consists mainly of gram-positive microorganisms, including coagulase-negative Staphylococcus, Corynebacterium spp., and anaerobes such as Propionibacterium (21). These resident organisms rarely cause foodborne illness. Unlike the resident organisms, transient bacteria colonize the superficial layers of a hand (8, 21, 39). Transient bacteria are often transferred to and from hands by cross-contamination from touching or handling raw foods or dirty surfaces; these transient bacteria frequently cause foodborne illnesses as well as nosocomial infections in hospitals (8, 9, 21).

Guzewich and Ross (18) studied 66 outbreaks that occurred in the United States between 1975 and 1998; they found that 82% of these outbreaks implicated food workers as the source of contamination and that hands were the
source of pathogen transmission in 34 (∼50%). Compliance with hand washing guidelines varies depending on hand washing training, ease of access to washing facilities, and workload (1, 16, 41).

Published research indicates that hand washes lasting longer than 20 s have little additional benefit but that washes for less than 10 s may not efficiently remove soil (26, 30). A quick wash (5 s) without soap has been commonly observed in busy environments (6, 15, 35, 41).

Drying hands is regarded as a crucial step in hand washing because moist surfaces transfer bacteria more readily than dry surfaces (14, 20, 25, 31, 38, 42), and drying is stipulated by the U.S. FDA Model Food Code (44). Paper towels dry hands quickly and can be used as a barrier to protect against recontamination from doorknobs and sink faucets (31, 36, 37), but they may not remove bacteria from palms and fingers as well as from fingertips (45). Recontamination of up to 1 log CFU per hand is possible by transfer from a jammed paper towel dispenser (19).

This research was undertaken to establish the importance of several keys factors, using methods that are robust, sufficiently replicated, and statistically valid. This study provides a quantitative measurement of the effectiveness of a minimal 5-s wash and a longer FDA Model Food Code–compliant hand wash (20 s) with and without food debris. The amount of bacteria removed by paper towels during hand drying was also quantified.

MATERIALS AND METHODS

Bacterial strain and growth conditions. A nonpathogenic nalidixic acid–resistant Enterobacter aerogenes surrogate for Salmonella (9, 28, 39) was grown overnight at 37 °C in tryptic soy broth containing 50 μg/ml nalidixic acid. Cells were harvested by centrifugation (Micro 12, Thermo Fisher Scientific, Waltham, MA) at 5,000 × g for 5 min and then were washed in phosphate-buffered saline (PBS; 0.1 M, pH 7.2). This process was repeated three times. The cell pellets were resuspended in PBS to form a solution of ∼8 log CFU/ml.

Inoculation solutions. The inoculation solution was created by serially diluting the harvested cells in PBS until a ∼6-log CFU/ml solution was formed. For the soiled-hand inoculation solution, 5 ml of the ∼6-log CFU/ml solution was added to 25 g of 80:20 ground beef purchased from a local supermarket in New Brunswick, NJ. The ground beef and bacteria solution were kneaded by gloved hands in a stainless steel bowl that had been sanitized with 60% ethanol. The resulting mixture was split into ∼5-g samples using a top-loading balance (Ohaus Corporation, Parsippany, NJ).

Participants. Twenty volunteers were asked to participate in the hand washing experiments. Participants were rejected if any open cuts or wounds were present on their hands, if they were ill or self-identified as immunocompromised, or if they were uncomfortable with any aspect of the experiment. Before each wash scenario, the participants were instructed to wash their hands with plain soap and dry them with paper towels. After the experiment, the volunteers were instructed to wash their hands, dry them with paper towels, and then apply hand sanitizer.

Quantification of E. aerogenes on hands. The glove juice method is a type of whole-hand measuring protocol that uses buffer inside a glove to recover the bacteria on a hand (3, 4). The glove juice method has been used in previous studies to determine the bacterial concentration on volunteers’ hands and has proven to be reproducible (5, 13, 22, 23, 32–34, 40, 43). Briefly, a nitrile glove (Fisherbrand powder-free nitrile examination gloves, Thermo Fisher Scientific) is filled with 20 ml of PBS. The loose-fitting glove is put over the volunteer’s hand, and the hand is massaged for 1 min. The glove is carefully pulled off, and the buffer is collected in a vial. The resulting solution contains the bacteria that were removed from the hand.

Hand washing scenario protocols. Several hand washing scenarios were studied in this experiment. Each experiment consisted of 20 replicates, each from a different individual. Each of the 20 individuals participated in each scenario once. The participants were given very basic instructions on how to wash their hands, and to reduce bias, only the time and drying method were communicated. With the exception of the 5-s wash, all volunteers were asked to wash their hands for 20 s with warm (18 to 35 °C) municipal tap water. Volunteers who used soap used 1 ml of unscented, plain liquid hand soap (Up and Up, Target Brand, Minneapolis, MN) to wash their hands. Volunteers’ hands were either air dried, without the aid of a mechanical dryer, or were dried using autoclaved paper towels (White Multifold Towel, Oasis Brand Inc., Winchester, VA). Volunteers participated in no more than one hand wash experiment per day.

The following scenarios were tested:

(i) Soap versus no soap on nonsoiled hands. Two 0.5-ml aliquots of the inoculation solution that contained ∼6 log CFU/ml E. aerogenes was placed in each hand of volunteers, and volunteers evenly dispersed the inoculum by rubbing their hands together. The hands were allowed to air dry until visibly dry (∼60 s) before continuing. A volunteer’s nondominant hand was sampled using the glove juice method to determine the bacterial concentration on that hand. This sample was used as the preswash bacterial concentration on the hands. After waiting for their nondominant hands to dry, the volunteers washed their hands once with plain soap, under running water for 20 s, and let their hands air dry. Wash time was measured using a timer. After the hands dried, the microorganisms were recovered using the glove juice method described above for both hands. These samples were used for the postwash bacterial concentration on the hands. The same scenario was repeated with the same individual, without soap, on a different day.

(ii) Soap versus no soap use on soiled hands. The volunteers followed the same protocols as in the previous scenario, except that, to inoculate their hands, volunteers picked up and spread 5 g of 80:20 ground beef inoculated with ∼6 log CFU/5 g E. aerogenes over their hands and waited 30 s. The ground beef remained visibly moist after it was spread on the hands. Scenarios with and without soap use were performed as above.

(iii) Paper towel versus air drying. The volunteers’ hands were inoculated with 1 ml of the inoculation solution (∼6 log CFU/ml E. aerogenes). After the volunteers’ hands were visibly dry (∼60 s), their nondominant hands were sampled using the glove juice method. This sample was used for preswash bacterial concentration on the hands. The volunteers then washed their hands, without soap, for 20 s under running water, and then dried their hands with paper towels. Volunteers were given one paper towel at a time to dry their hands until they felt that their hands were sufficiently dried. No volunteer used more than two paper towels. Each paper towel was collected and put in a Whirl-Pak 7-oz (207-ml) sterile filter bag with 25 ml of buffer. The paper towel...
and buffer were then homogenized using a stomacher (Dynatech Laboratories, Alexandria, VA). The homogenized samples were plated onto agar to determine the bacterial concentration on the paper towels. After the wash and the drying, both hands were sampled using the glove juice method. These samples were used for the postwash bacterial concentration on the hands.

(iv) Minimal (5-s) wash. The 5-s wash followed the same method as described above, except that the hands were only washed without soap and for 5 s. The effects of soap and debris were not studied for the 5-s wash.

Bacterial quantification and data analysis. After the washing scenarios were completed, the samples collected were serially diluted with PBS and were plated onto MacConkey agar (BBL, BD, Franklin Lakes, NJ) with 50 μg/ml nalidixic acid added. The plated samples were incubated overnight (18 to 24 h) at 37°C. The CFU were counted the next day to enumerate the bacterial concentration on the prewash hands, postwash hands, and on the paper towels used for drying. All counts were expressed as CFU per hand or per paper towel.

The prewash concentration was determined by taking the arithmetic count from the nondominant hand and multiplying by 2 (to estimate the concentration on both hands). The log reduction was determined by taking the difference between the logarithm of the estimated prewash concentration and the logarithm of the sum of the postwash concentration on both hands.

A frequency histogram of the data was assembled using Excel (Microsoft, Redmond WA) for each scenario. The frequencies for each wash scenario were plotted to visualize variability in log reduction rates and to compare the different washing scenarios. The frequency represents the number of times a specific log reduction was observed. A paired t test using Excel was used to determine significant differences between samples. A P value less than 0.05 was considered significant. When more than two comparisons were being made, an analysis of variance and a Tukey’s range test (MATLAB, Natick, MA) were used to determine whether multiple means were significantly different at the 0.05 level of significance.

RESULTS

Although washing hands for 20 s with soap is the recommended practice, studies that observed hand washing in normal practice suggest that most people wash hands for considerably less time (6, 15, 35, 41). Figure 1 shows a frequency diagram comparing minimal hand washing with Model Food Code hand washing, where the y axis shows the frequency, or number of observations corresponding to a given log reduction on the x axis. Our results show a statistically significant difference (P = 0.003) between the reduction of the inoculated E. aerogenes that was achieved using the FDA Model Food Code recommended wash (20 s, with soap) and air drying and that achieved using a minimal wash (5 s, no soap) with air drying (Fig. 1). The recommended wash had an average reduction of 1.7 ± 0.8 log CFU, and the minimal wash had an average reduction of 1.0 ± 0.4 log CFU. The greater variability in the 20-s wash time with soap is also apparent from Figure 1.

Four separate washing regimes were compared in Figure 2: washing hands for 20 s, without soap and with no debris added to the hands; washing hands for 20 s, with soap and with no debris; washing hands for 20 s, without soap and with ground beef debris on the hands; and washing hands for 20 s, with soap and with ground beef debris. The reductions observed ranged from no observed reduction to ~4 log CFU reduction (Fig. 2). The least log reduction was seen when no soap was used with ground beef debris on the hands (1.1 ± 0.6 log CFU reduction). The next greatest log reduction was seen when no soap was used and no debris was present on the hands (1.4 ± 0.4 log CFU reduction), followed by that seen when hands were washed with soap and without debris present (1.7 ± 0.8 log CFU reduction). The greatest log reduction was observed when soap was used and debris was present on the hands (2.2 ± 0.5 log CFU reduction). There was only a slight difference in the effect of ground beef debris on the hands when soap was not

![FIGURE 1. Reduction of Enterobacter aerogenes, comparing a minimal hand wash (5-s wash, no soap; ●) and the U.S. FDA Model Food Code–recommended wash (20-s wash, with soap; ○). In both scenarios, the hands were air dried.](http://meridian.allenpress.com/jfp/article-pdf/1688243/0362-028x_jfp-14-245.pdf)

![FIGURE 2. Reduction of Enterobacter aerogenes, comparing a 20-s hand wash with (circle) and without (triangle) soap, and with (closed) and without (open) debris, from least to greatest average log reduction: no soap, with debris (▲), no soap and no debris (△), soap and no debris (○), and soap, with debris (●).](http://meridian.allenpress.com/jfp/article-pdf/1688243/0362-028x_jfp-14-245.pdf)
used (0.3 log CFU difference in reduction), and this difference was not significant. Similarly, the effect of using soap when no debris was on the hands was slight (0.3 log CFU difference in reduction), and this difference was not significant. When the two soap treatments (ground beef debris and no debris) were compared, the difference was significantly greater (0.5 log CFU difference in reduction; \( P < 0.01 \)), with the greater mean reduction observed when ground beef debris was present on the hand. Statistically significant differences were also observed between other treatments, with the greatest difference (1.1 log CFU difference in reduction) seen when ground beef was present on the hands; this was the case whether soap was used or not, although the greater reduction was seen when soap was used.

The effect of using paper towels to dry hands after washing or letting the hands air dry (i.e., evaporation) on the frequency of log reductions of \( E. \) aerogenes achieved per wash is shown in Figure 3. Using a paper towel to dry hands resulted in a 1.9 \( \pm \) 0.9 CFU per wash reduction of \( E. \) aerogenes, which was a significantly (\( P = 0.03 \)) greater reduction than that achieved with air drying (1.4 \( \pm \) 0.4 CFU per wash reduction). The greater person-to-person variability seen when paper towels are used is apparent from the wide spread seen in the paper towel data in Figure 3 as well as the standard deviations reported above.

Figure 4 shows the amount of \( E. \) aerogenes (log CFU per towel) recovered on the first and second paper towels used by study participants to dry hands after the 20-s washing regime, without soap. One of the first 20 towels used was below the \( E. \) aerogenes detection limit (2.0 log CFU per paper towel). Five of the second group of towels used had bacterial concentrations below the detection limit (2.0 log CFU per paper towel). Five of the second group of towels used had bacterial concentrations below the detection limit (2.0 log CFU per paper towel). Three volunteers did not use a second paper towel. The mean log CFU per towel for the countable first paper towels used was 3.8 \( \pm \) 0.6 log CFU per paper towel and for the countable second paper towel used was 3.5 \( \pm \) 0.6 log CFU per paper towel.

**DISCUSSION**

Our results manifest a greater variability in log reduction for a 20-s wash time with soap versus a 5-s wash time with no soap (Fig. 1). If there is person-to-person variability in hand washing technique and effectiveness, it logically follows that this effect is smaller when duration is shorter, but as wash duration lengthens (and as soap is added), the variability will increase. We have previously observed less variability for an intervention with hand sanitizer versus hand washing (39), which may be because sanitizer effectiveness depends less on technique than hand washing effectiveness does. Clearly, more research on the possible causes of person-to-person hand washing variability is needed.

Our results show no significant difference between washing for 20 s with or without soap when no debris is present on the hands (Fig. 2). This is in contrast to a study by Coates et al. (10), who examined the reduction of \( Campylobacter \) on the fingertips when using rinses with and without soap. These authors concluded that a wash with soap and water was more effective at removing \( Campylobacter \) than a wash with only water, but they did not do a statistical analysis or report standard deviations. Although our results show that the average log reductions are not significantly different whether or not soap is used when there is no debris on the hand, the 1.1 log CFU greater average effectiveness of soap was statistically significant (\( P < 0.05 \)) when ground beef was present. Although the reasons for this are unclear, we speculate that, because the individuals can see and feel the ground beef on their hands, they are more effective in their hand washing technique when trying to remove it. Soap adds to this effectiveness because of its surfactant properties, allowing the insoluble ground beef particles to become soluble in water and then to be rinsed away.

Two separate studies, Michaels et al. (26) and Courtenay et al. (11), used inoculated ground beef as debris and found...
log reductions of an inoculated surrogate similar to those in our study. Michaels et al. (26) reported a 1.5- to 2.5-log CFU reduction of Serratia marcescens, with a 15-s wash using antimicrobial soap; Courtenay et al. (11) observed a 2.7-log reduction. This is consistent with our study (Fig. 2), which shows an average log reduction of 2.2 log for a wash with soap and debris on the hand, with individual hand washing effectiveness varying from a low of a 1.1-log reduction to a high of a 3.0-log reduction.

A study by Gustafson et al. (17) used 99 volunteers and tested four different methods of drying hands and their effect on the microbial reduction of bacteria during a hand wash. They tested cloth towels from a rotary dispenser, paper towels in a stack, a forced air dryer, and air drying (evaporation). Although these researchers indicated that there was no difference between the drying methods examined, they reported their data as differences in CFU rather than differences in log CFU. If the correct statistical transformation (logarithmic) is used on the reported data and a log reduction is calculated, it can be inferred that drying hands with paper towels provides a 0.5-log CFU greater reduction than evaporation (air drying) or drying with warm air. Similarly, Coates et al. (10) determined that Campylobacter is more readily removed from fingertips if a paper towel is included in the hand washing regime. These studies agree with our finding (Fig. 3) that using a paper towel provides a statistically significant greater log reduction (1.9 ± 0.9 log reduction) versus air drying by evaporation (1.4 ± 0.4 log reduction).

A minimal hand wash (5 s, no soap) can reduce bacterial populations on the hands by 90%, but an FDA Model Food Code–compliant hand wash (20 s, with soap) is significantly more effective. When hands are not contaminated by food debris, our results show that a 20-s hand wash is equally effective with or without soap. Soap is more effective when debris is present on the hands, likely because of the effect of the soap in removing debris, and perhaps by the sensory cues from the presence of ground beef. Paper towels appear to offer a measurably significant benefit (i.e., 0.5 log CFU greater reduction) when used after hand washing. Used paper towels provide a 0.5-log CFU greater reduction (1.9 ± 0.9 log reduction) versus air drying by evaporation.

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